A STUDY OF THE SEED ECOLOGY OF TWO SPECIES OF KOWHAI *Sophora microphylla* and *Sophora prostrata* IN CANTERBURY, NEW ZEALAND

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ABSTRACT

The seed ecology of *Sophora microphylla* (common kowhai) and to a lesser extent *Sophora prostrata* (prostrate kowhai) from two Canterbury sites was studied. The aspects of the seed ecology examined were firstly, immature germination, embryo maturity and seed predation in *S. microphylla* followed by a study of the seed banks, seed fall, seed dispersal, the influences of temperature and light on germination and early growth, and natural mechanisms employed for the scarification of the seed coat in both *S. microphylla* and *S. prostrata*. Germination experiments revealed that *S. microphylla* seeds can germinate when they are immature and that embryo maturity is achieved in most seeds at around 16 weeks of age, although some mature in only 12 weeks. The only significant predator of *S. microphylla* seed was the larvae of the moth *Stathmopoda aposema*, which began to predate the seeds at about 8 weeks of age and continued to predate them from this time on. It was found that predation by this organism accounted for the death of about 28% of seeds in the population of trees studied in the 1991-92 year, although annual fluctuations probably occur. Wind was found to be the principle mechanism of seed removal from the trees of both *S. microphylla* and *S. prostrata* and the main, if not the only, dispersal mechanism in *S. prostrata*. Although wind does play a role in the short distance dispersal of *S. microphylla* seeds (particularly the whole fruit or pod) the chief mechanism of long distance dispersal in this species is water. *S. microphylla* possesses both buoyant (up to 27%) and non-buoyant seeds, attributable to the density of its embryo, which is thought to aid the long distance dispersal process. It was found that *S. microphylla* possessed two types of seed banks one on the tree (resulting from the retention of previous seasons seed) and the other in the soil. The soil seed bank was found to be extensive and deep although most seeds were contained in the top 10cm of the soil profiles dug. *S. prostrata* seemed to have no long-term seed banks as such although many seeds are assimilated into the humus layer beneath parent plants before being lost downslope or germinating, indicating a very temporary soil seed bank at most. The seeds of both *S. microphylla* and *S. prostrata* were sensitive to temperature when germinating and have an optimum germinating range of about 10-20°C. As expected there was no obvious sensitivity to light during the germination of either species and most seeds established more successfully after burial (lightless conditions). However, the light intensity appeared to affect young seedlings with both species showing a distinct etiolation pattern. The scarification of seeds in nature appeared to be different for each species. For *S. microphylla*, scarification of the seed coat did occur in the soil and this was supported by the discovery of germinated and imbibed seeds there. But how this happens is still not clear. It is thought that it may be a result of very gradual microbial breakdown of the micropyle, or influences from other soil organisms. It is also possible that many seeds are scarified in river systems by alluvium in river beds, however experiments showed that many seeds particularly those which are not buoyant may be destroyed in the riverbed environment. Mature *S. prostrata* seeds when relatively young appeared to have soft seed coats, and at this stage seeds would imbibe and germinate without scarification. As seeds get

progressively older however, the seed coats harden and scarification was necessary before germination could take place. After hardening, breakage of the seed coat was thought to occur due to abrasion of soil particles as the seeds move downslope under the influence of gravity or by similar mechanisms to *S. microphylla* after assimilation into the soil.

CHAPTER 1

INTRODUCTION

1. General introduction and scope

This project examines the seed ecology of two species of kowhai, *Sophora microphylla* and *Sophora prostrata* (Leguminosae). It includes research on the ecological significance of seed maturity, germination characteristics, dispersal and external influences such as seed predation and fire.

Worldwide research in seed ecology has not been extensive compared with other ecological disciplines. Much of the work on seeds has concentrated on the physiological and biochemical changes during maturation and germination, rather than the ecological significance of seed physiology and external influences on seeds. Often, in areas where floras are diverse, such as New Zealand, there is even less detailed knowledge about the seed ecology of specific species, with many studies concentrating on a specific attribute of a number of different species, rather than the comprehensive study of only one. Thus, for many New Zealand species, virtually nothing is known about their seed ecology and *Sophora microphylla* and *Sophora prostrata* are no exception to this. Putting this in perspective, a search of the literature by Fountain and Outred (1991) on the germination requirements of New Zealand native plants, revealed information in varying detail on only 113 species, some 5% of the estimated total vascular plants in New Zealand, most of them from forested habitats.

Much of the specific work done on New Zealand seeds concentrates on a few species, usually of agronomic importance, especially grasses, such as *Triticum*, *Hordeum*, *Zea* and *Oryza* (Poaceae) (Fountain and Outred 1991). This work has incorporated an array of physiological and ecological studies including recalcitrance, vivipary, quiescence, dormancy, after ripening, inhibitors, light and temperature (although studies on dormancy, after ripening and inhibitors are sparse).

Very little about the seed biology of *Sophora* is known. The main research of substance has been carried out on *S. microphylla* seeds, presumably because it is the most extensive and widespread species. Dr Eric J. Godley is the foremost authority on this plant and has studied its seed and fruit in some detail. Much of his work has centred on the dispersal of the seeds, particularly by water (Sykes and Godley 1968, Markham and Godley 1972, Godley 1975), although he has researched some of the physiological and developmental attributes of the seed (Godley 1975, 1982). Sykes and Godley's 1968 study of transoceanic dispersal in *S. microphylla* seed has led to a small number of genetic and taxonomic studies. Murray and Porter (1980) for example, found that *S. microphylla* from New Zealand was the progenitor of the Chilean *S. microphylla* as well as another Chilean species *Sophora macrocarpa* which grows inland from its coastal margins. The copious seed production and extensive soil seed banks of *S. microphylla* has led to its mention in some seed bank studies (eg. Partridge 1989).

Sophora prostrata is almost entirely devoid of any specific research. Apart from its apparent ability to hybridise with *Sophora microphylla* (Metcalf 1972), it is seldom mentioned in the literature. Because of its contrast to *S. microphylla* in a number of characteristics, it was felt that this species would provide an interesting comparison in this project.

2. Brief Description of *S. microphylla* and *S. prostrata*

I *Sophora microphylla*

Sophora microphylla is probably the most common and widespread of the *Sophora* species in New Zealand. It is native to New Zealand but is also found in Chile and on Gough Island in the South Atlantic (Metcalf 1972). It is described as a tree, although smaller mature specimens could be described as large shrubs. It reaches a height of 10m with a trunk up to 60 cm in diameter (Allen 1961). Plate 1 shows one of the mature *S. microphylla* specimens used in this study.

Plate 1. A mature *Sophora microphylla* specimen.

An interesting aspect of *S. microphylla* is that it often, but not always, has a divaricating juvenile stage (Godley 1975, Metcalf 1972, Allan 1961). It seems that the extent of divarication differs greatly between individual plants and within individual populations. Godley (1975) noted that in populations on the west coast of Auckland (*S. microphylla* var. *fulvida*), in the Wanganui river catchment in north-west Nelson (*S. microphylla* var. *longicarinata*) and on the Chatham Islands (*S. microphylla* var *chathamica*), young *S. microphylla* usually grow straight into trees and flower in as little as 4 years. However, populations with a weak expression of the juvenile form, for example many North Island races, may not flower for up to 7 years and many with a strong expression such as those on the east coast of the South Island, may take as much as 15 years. From my own observations, the extent and timing of divarication varies between individuals raised from the same seed source. Some plants may show signs of divarication in as little as 8 weeks after germination while others from the same seed source did not show divarication until they were 8 months of age. Plate 2 shows a *S. microphylla* plant at only 12 weeks of age (from germination) showing distinct signs of divarication.

Plate 2. Divarication in *Sophora microphylla* as seen 12 weeks from germination (about actual size).

Although the exact significance of the divarication is not known, Godley (1975) believes that this could be a result of a recent hybrid origin of the species with the parents being *S. prostrata* and *S. tetraptera.* Although they don't do so now, the ranges of these species overlapped in the past when the islands of New Zealand were joined during the glacial period. Godley (1975) also notes that the divaricating juvenile form of *S. microphylla* may have been derived from *S. prostrata*, and races could have segregated out from the original hybrid with differing degrees of expression of this character. In New Zealand, *Sophora microphylla* grows in open forests, forest margins, along rivers and in open places in lowland and mountain country through the North, South and Chatham Islands. Altitudes at which it is found vary from sea level to 800m. Flowering times vary considerably. In Canterbury most trees flower from August to November, as also at most localities throughout the country (Godley 1975), although some trees have been seen flowering on the Plains in July, and Metcalf (1972) noted others on Banks Peninsula which flower in early June. Flowering times seem to be a factor of genetic variation between individuals and not a

result of environmental conditions or latitude. Canterbury, for example, has some of the earliest flowering individuals in New Zealand (Godley 1975).

Godley (1975) noted that in *S, microphylla*, small, young inflorescences are already visible in mid summer and gradually increase in size throughout the remainder of the year until the flowers open. He also states that exceptional trees may flower regularly in the autumn and certain early flowering races, depending on the season, may come into flower by July. Plate 3 shows the flowers and foliage of *S. microphylla.*

Plates 3A and 3B. The (A) flowers and (B) foliage of *Sophora microphylla* (after Salmon 1980). They are about natural size.

(A)

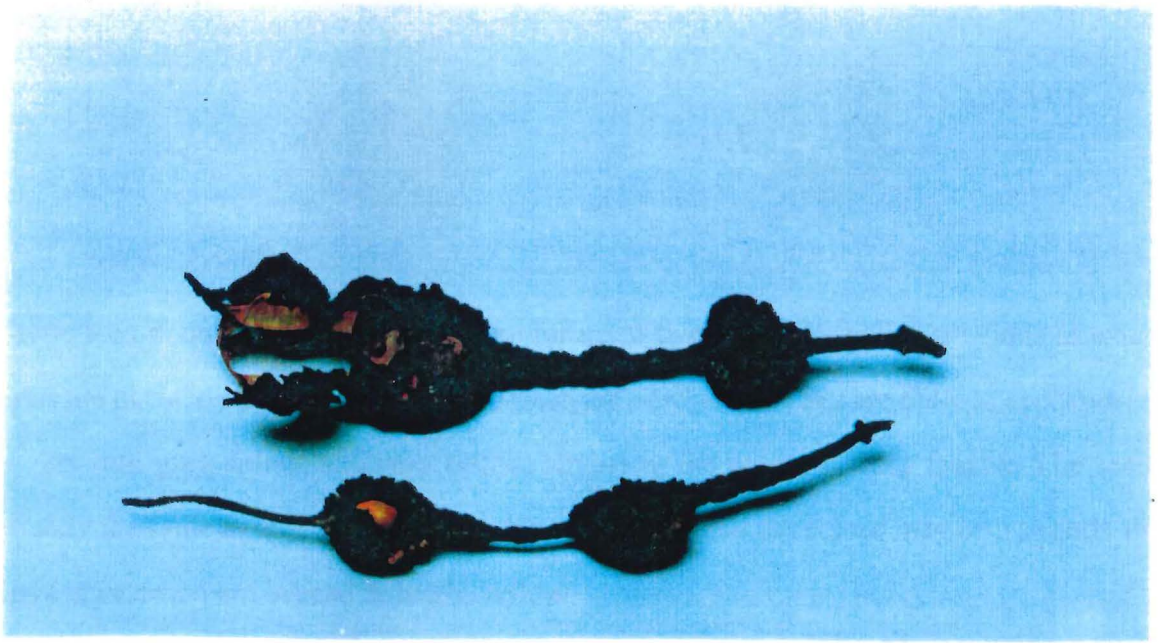
(B)

Allan (1961) describes *S. microphylla* as having pinnate leaves, up to 15cm long with 20-40 pairs of obovate-oblong leaflets and a terminal leaflet. The leaflets are 5-7mm long and younger leaflets have appressed hairs which they lose as they get older. The flowers are about 45mm long and pale yellow. The 10 stamens are free (Godley 1982). The trees are evergreen maintaining a uniform amount of foliage throughout most of the year, but some individuals lose most of their leaves just before and during flowering (personal obs). Godley (1975) also noted the deciduous nature of some populations with many of those in the north being well known for flowering when their branches are bare. A tendency for deciduousness is seen throughout the North Island

and on the West Coast of the South Island but individuals growing in Canterbury and Otago are generally evergreen and maintain most of their old leaves while producing young ones (Godley 1975). It is thought that this occurs due to the inevitably large allocation of resources to the large flowering and seeding effort.

The juvenile fruit, or pods, appear soon after fertilisation, are green, 7-10mm long and 3-5mm wide. They progressively elongate to full size (about 12cm for a 6 seeded pod). After this, the pod dries, turns brown and becomes brittle at which time disintegration takes place releasing the seed. Plate 4 shows the disintegration of *S. microphylla* seed pods in progress.

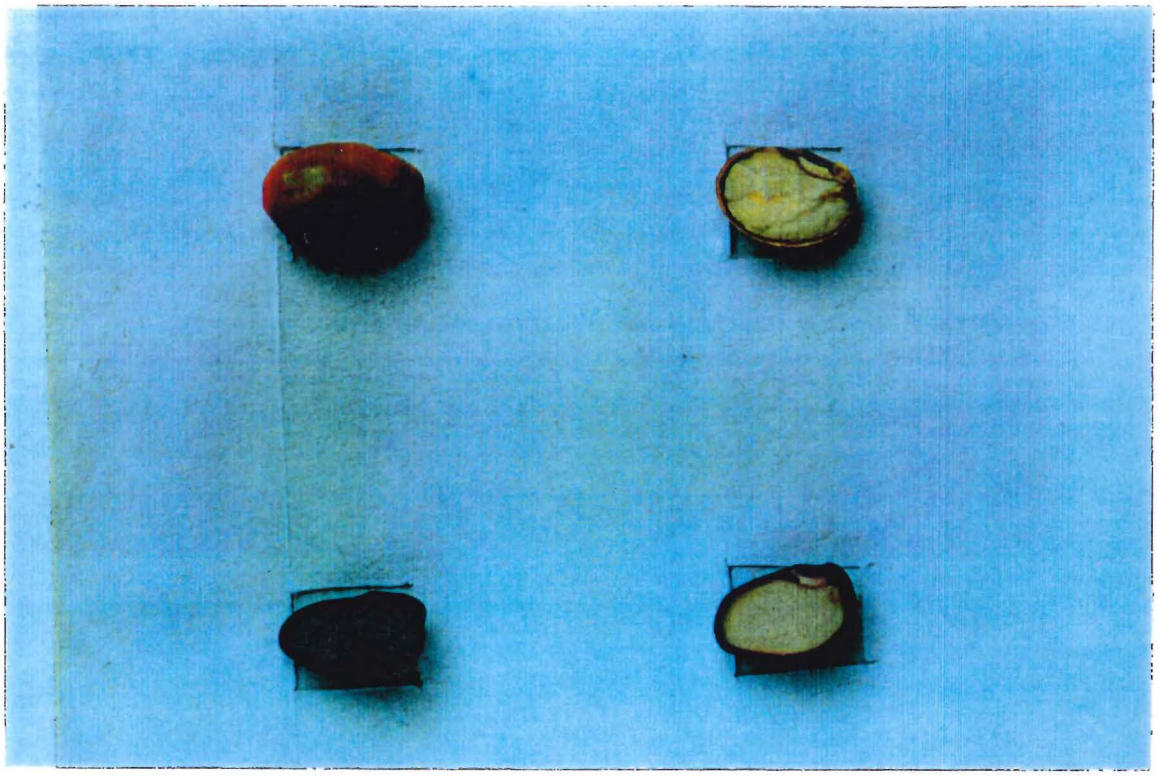
Plate 4. Disintegration of *Sophora microphylla* seed pods. The pods are about actual size. Note the ribbed outer coat of the pods.



The seeds develop within the pod, in separate seed cavities. They are green at first, and turn fully yellow at about 14 weeks. They dry out and mature by 20 weeks of age, after which they have developed a hard seed coat impenetrable to water, allowing them to remain dormant in soil seed banks for a long period of time. The number of seeds per pod varies greatly, from as many as 15 to as few as 1. In general however, there tend to be between 3 and 6 seeds per pod. The seeds are

5-8 mm long, 3-5mm wide and bright yellow at first but turning more of a mustard colour with age. The seeds of both *S. microphylla* and *S. prostrata* magnified to about twice normal size are shown in Plate 5.

Plate 5. The seeds of *Sophora microphylla* and *Sophora prostrata* at about 2x magnification.



Some seed pods are retained on the tree for up to two years. Visually, 'previous season's' seed pods are distinctly different from those which are newly ripe. They are older in appearance, being a very dull grey colour and very brittle, almost to the point of being fragile. The seeds look exactly the same as mature 'new season's' seed, except that the colour may be a slightly different light mustard brown instead of bright yellow.

II <u>*Sophora prostrata*</u>

Sophora prostrata differs markedly from *S. microphylla*, most notably in habit, habitat, and the structure of its seed pod. The plant is a much branched, prostrate or bushy shrub, up to approximately 2m. The branches are densely divaricating, and often tightly interlaced. This

characteristic becomes noticeably greater with altitude, or exposure to wind (personal obs). A mature *S. prostrata* specimen from the Canterbury Plains is shown in Plate 6.

Plate 6. A mature *Sophora prostrata* specimen.

S. prostrata is much less common and has a much more restricted distribution than *S microphylla* (Metcalf 1972). It grows in grassland and rocky places in lowland and mountai regions east of the main divide, in the South Island, from Marlborough to South Canterbury from 80 to 800m above sea level (Metcalf 1972).

The structure of the foliage is very similar to that of *S. microphylla* but the leaves are substantiall smaller. They are pinnate, reaching a maximum length of 50mm in some cases, but seldon exceeding 30mm. The leaves have 2-5 pairs of oblong to oblong-ovate leaflets 3-5mm long. Th plant is evergreen and does not appear to lose its foliage in the same way *S. microphylla* doe during flowering.

Flowering was noted by Metcalf (1972) to occur in Christchurch from late October to mic November. However, plants on Banks Peninsula have been seen flowering in early February and immature unripened seed have been seen occurring at Dyers Pass (Banks Peninsula) as late as early June (personal obs). A feature distinguishing the form of *S. prostrata* from that of othe species is the bent pedicels, so that the flowers hang 'upside down' (Metcalf 1972). The flower and foliage of *S. prostrata* are shown in Plate 7.

Plates 7A and 7B. The (A) flowers and (B) foliage of *Sophora prostrata*. After Salmon (1980) They ar about actual size.

(A)

(B)

The flowering times of *S. microphylla* and *S. prostrata* do overlap and the evidence for this is th presence of natural hybrids between the two species, examples of which have been seen at Dyer Pass on the Port Hills. *S. prostrata* flowers range from bright canary yellow to deep orange.

In contrast to *S. microphylla*, the pods or fruit of *S. prostrata* are smaller, lighter, lack ribs an contain fewer seeds. Most pods will contain only 1 or 2 seeds, although 3 seeded pods ar reasonably common and some 4 seeded specimens have been found on the Port Hills. Th material of which the pod is made is of lighter construction than that of *S. microphylla* bu matures in a similar fashion and in approximately the same time. The pod is grey-green at fir and then becomes dark grey-brown.

The seeds of *S. prostrata* are green at first, but then become a moderate to dark reddish brown, about 3-6mm long and 2-4 mm wide. They develop a hard impenetrable seed coat with age, although this characteristic seems to develop more after seed maturity, unlike *S. microphylla*. Some apparently ripe seed can have a relatively soft seed coat, and will be penetrated by water. This is discussed in Chapter 8 in more depth. Refer to Plate 5 (p9) for the photograph of *S. prostrata* seeds.

3. Some of the problems and features of the reproductive biology of *S. microphylla* and *S. prostrata*

At the beginning of this project some features of the reproductive biology of both species were identified and became a foundation for the research.

* The most characteristic feature of both species, although more particularly *S. microphylla*, is the tough impenetrable seed coat or testa. This is found extensively among legumes (Mayer and Poljakoff-Mayber 1982). The presence of the hard testa poses the question of how these seed coats are broken in nature, allowing germination and plant recruitment to take place. There are a variety of possible mechanisms including mechanical abrasion in the soil and riverbeds, chemical degradation, fire, digestive tracts of animals and birds and microbial activity.

* Seed predation is a problem encountered by both species although it is more prolific in *S. microphylla*. It has serious implications for sustaining viable seed populations. Very little is known about the numbers and types of predators, especially potential introduced predators such as rodents, and the extent to which they predate the seed.

* *S. microphylla* in particular has some interesting, if not unusual characteristics. For example, toxic phenolic compounds have been isolated from the seeds, nectar and wood. The nectar causes narcosis in what were thought to be primary pollinators such as native birds (Connor 1981), perhaps suggesting the existence of an immune, species specific pollinator.

NOT IN REFERENCES !!

* Both *S. microphylla* and *S. prostrata* establish on dynamic and open sites such as river terraces and broken bluffy slopes. The introduction of vigorous exotic weeds such as gorse, broom and introduced grasses, as well as intensive grazing and agriculture has possibly reduced their ability to recruit new individuals into existing populations.

4. Study aims and objectives

The main aim of this study was to investigate, quantitatively, the basic aspects of the seed ecology of *S. microphylla* and *S. prostrata* including germination characteristics of mature and immature seeds, influences of temperature and light, seed predation, seed production, dispersal, seed banks and dormancy mechanisms. It also attempts to address the overall ecological significance of these aspects, and how this may have changed since the, pre-Polynesian era. This will hopefully give an understanding of the life and fate of the seeds in *S. microphylla* and *S. prostrata* seed crops from the time of seed set to the establishment of new seedlings.

CHAPTER 2

STUDY SITES

1. General introduction

This chapter describes the location of study sites and briefly outlines the present climate, soil characteristics, and vegetation history of the region at each respective site. It was considered important that these aspects were included as they are relevant to the ecology of the plants under investigation.

The *S. microphylla* and *S. prostrata* plants, from which seed for this study were taken, are from natural populations of plants whose environment is likely to have been modified over the course of time. This has happened mainly through human activity and natural environmental changes including those triggered by catastrophic events (eg. fire). These modifications have probably not changed the basic soil composition of the study sites, but have almost certainly had drastic effects on the vegetation.

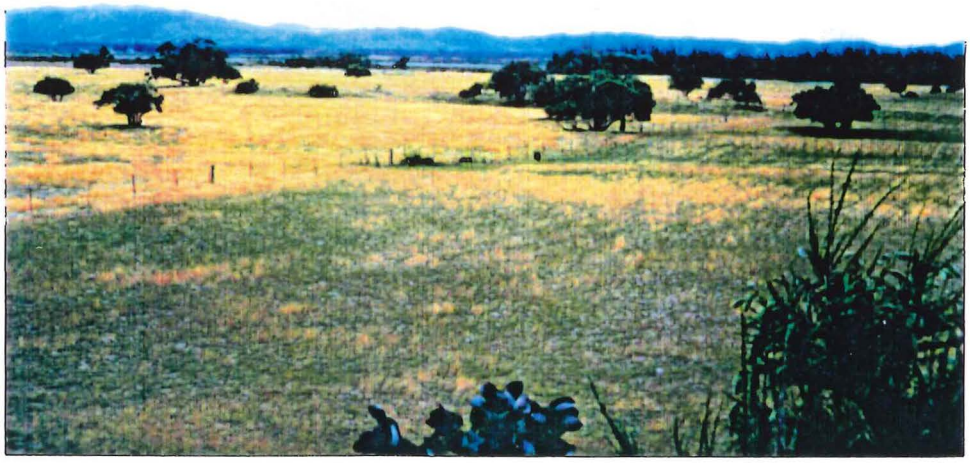
2. Location of Study Sites

In choosing sites to be used in this study a range of criteria were employed. Firstly, sites had to be located in reasonably close proximity to Christchurch in the interests of keeping costs down, and also because they would be visited on a regular basis throughout the year. The other criteria for choice of sites was that they had to be as close as possible to original natural populations of plants in the Canterbury region. This was relatively easy to achieve despite the fact that the environment surrounding these populations had been extensively modified.

Two main sites were used in this study; Site 1 (Grid Reference: 464497 NZMS260, Sheet L35. Scale 1:50 000), containing chiefly *S. microphylla*, and Site 2 (Grid Reference: 814333

NZMS260, Sheet M36. Scale 1:50 000), containing chiefly *S. prostrata*. Site 1 is located on the lower Waimakariri river terraces at Courtenay, approximately 30 km west of Christchurch at an altitude of approximately 160m above sea level. It is on the property of Mr D.J. Dillon, a sheep farmer of the area. Access to this site is by way of the Old West Coast Road from Christchurch and then by following an anglers access road located 3km before reaching Courtenay Domain Plate 8 shows some of the *S. microphylla* trees at Site 1.

Plate 8. *Sophora microphylla* trees at Site 1.

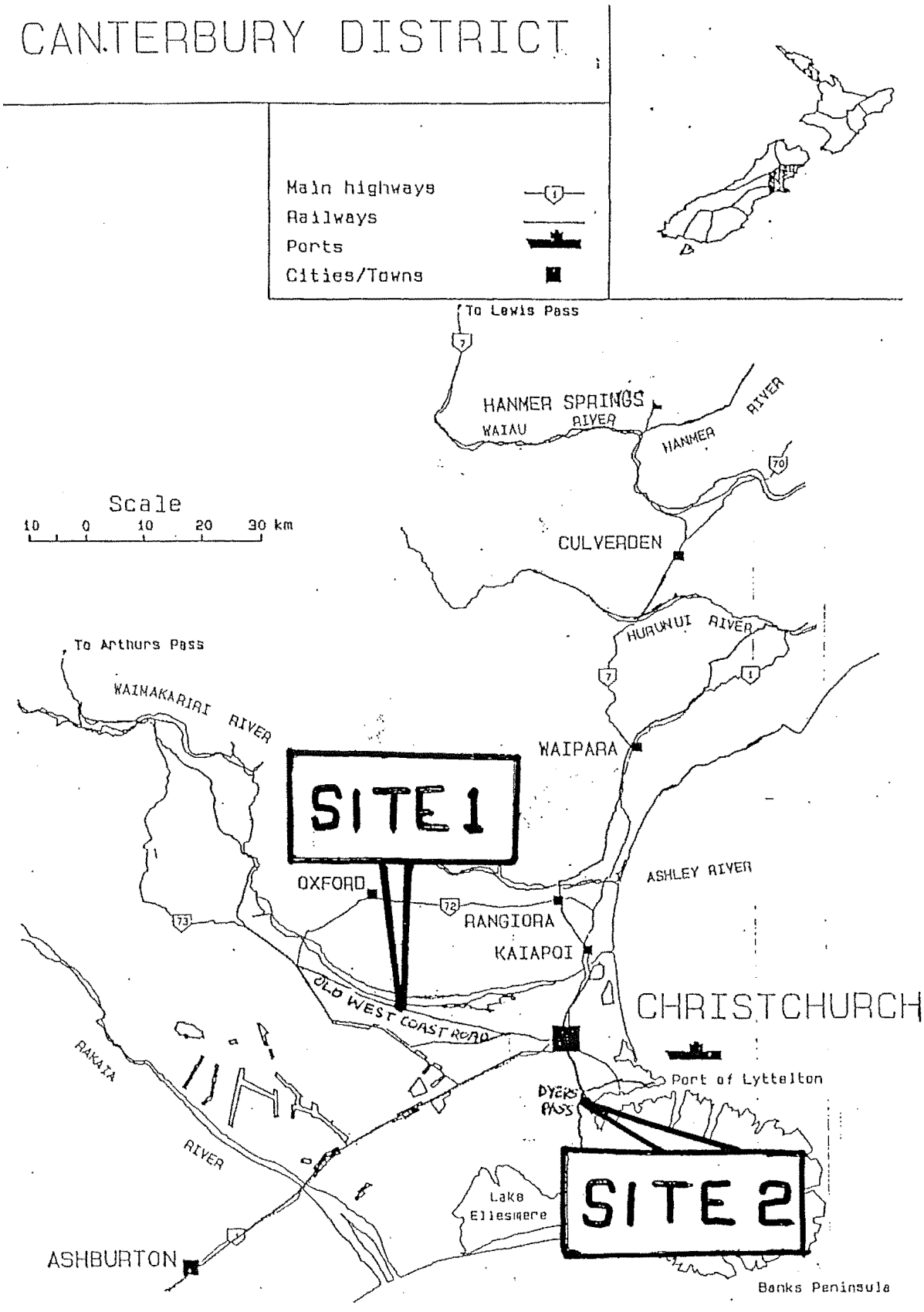


Site 2 was very different and although *S. microphylla* do grow in this locality there are only tw trees in the immediate vicinity. This site was located on the Port Hills of Banks Peninsula behin Sign of the Kiwi on the junction of Dyers Pass Road and Summit Road at an altitude c approximately 350m above sea level. The location is very accessible, due to nearby walkway: Plate 9 shows some of the *S. prostrata* population at Site 2.

Plate 9. *Sophora prostrata* plants at Site 2 (large green masses).

The locations of both study sites relative to the rest of Canterbury are given in Figure 1 on th following page.

Figure 1. The location of study sites.



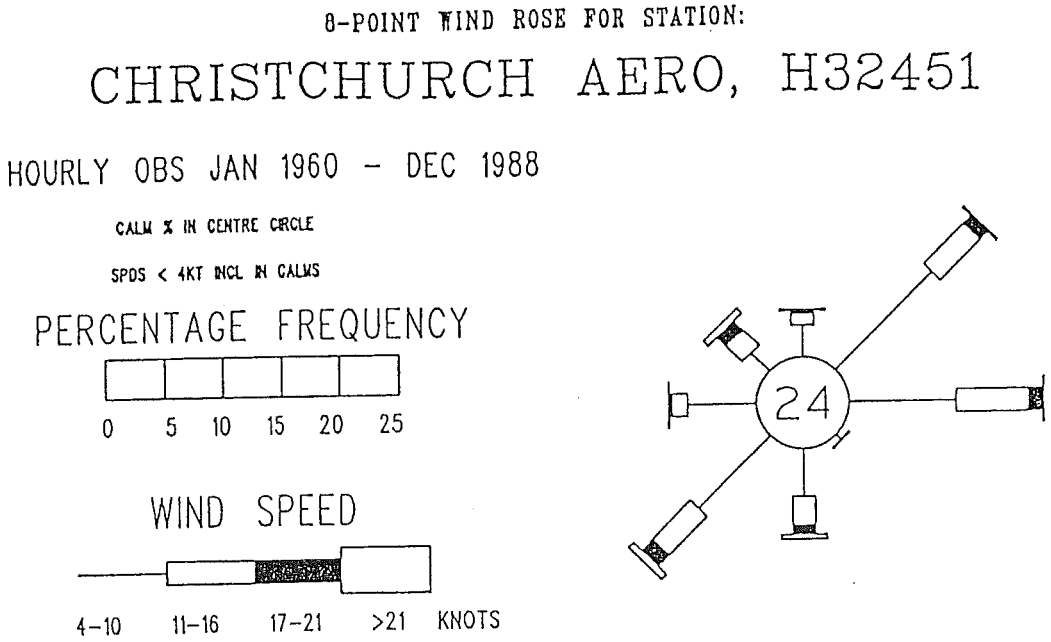
3. Description and History of Study Sites

Site 1. Site 1 is on the Canterbury plains and approximately 2ha in size. Although the topography and soils of Site 1 have not been extensively modified, the vegetation has. This is because it has been turned into farmland for the facilitation of grazing sheep and cattle which is characteristic of most of the Canterbury Plains today.

Being on the most recent river terrace system, Site 1 consisted primarily of gravels at the rivers edge with the associated yellow brown shallow and stony soils on the lower terraces formed from the build up of wind blown loess and deposition of river sediment. The terrace is approximately 200m wide with the majority of the 59 *Sophora microphylla* trees located 100-150m from the rivers edge. A single mature individual of *S. prostrata*, about 2.1m tall was also found at this site on the incline between the first two terraces. The ages of the *S. microphylla* trees at Site 1 were hard to assess and it was thought that many of the trees differed in age. It is estimated that the majority of trees were between 30 and 50 years of age, although some very large individuals, such as the one shown in Plate 1 may be as old as 70 years. These ages were estimated from the sizes of Christchurch trees whose ages are known.

The climate at Site 1 is characteristic of the majority of Canterbury, with weather coming from predominantly a westerly quarter due to the incidence of cyclones and frontal systems on the western seaboard of the South Island (de Lisle 1969). The characteristic northwest fohn winds, as they are termed, are strong and warm with low humidities, less than 45% (Molloy and Ives 1972). They tend to be more frequent in the autumn and spring equinoxes corresponding to maxima's in the strengths of the westerlies at these times. However, although they are the strongest winds in Canterbury, the northeast and southwest directions have the greatest frequency. Figure 2 is a wind rose showing the relative frequencies and strengths of wind direction at Harewood (Christchurch airport). Harewood has very similar climatic characteristics to Site 1.

Figure 2. Wind rose for Christchurch Airport (Harewood) January 1960-December 1988.



With weather coming from a predominantly westerly direction, the Southern Alps have an orographic effect on rainfall, trapping much of the moisture before it reaches the plains. Consequently site 1 is relatively dry with falls of 800mm to 1000mm (Ministry of Works and Development 1983). Heavy falls can occur on the plains and foothills, but this is usually only associated with a depression centred over the area, or just off the east coast (de Lisle 1969). The north west conditions often mean large falls in the catchment areas of the Alps, which can cause large scale flooding in the Canterbury river systems. Being on the lowest terraces of the Waimakariri river, Site 1 is very prone to this type of flooding.

During the summer, from October to March, maximum temperatures are high at Site 1, up to 36°C. In contrast to this the March to October period is more humid and cooler with minimum

temperatures as low as -14°C and frequent frosts (Molloy and Ives 1972). The sunshine hours at Site 1 are approximately 1900-2100 hours. Climatological data at Christchurch (Harewood) over the last 5 years, is summarised in Table 1.

Table 1. Christchurch climatological conditions at Harewood 1987-91.

Year	Total Annual Rainfall (mm)	Total Annual Sunshine (hours)	Mean Annual Temperature (C)
1987	584	2109	12.1
1988	303	2366	12.3
1989	707	2092	12.3
1990	542	2294	12.3
1991	638	2029	11.5

Source: Metservice, Christchurch Office (Harewood)

Vegetation history. The arrival of the Polynesians between the 10th and 15th centuries, and then the Europeans in the 1830's and 40's caused drastic modifications to the vegetation in Canterbury (Molloy 1969, Johnston 1969, Wardle 1991). The Pre-Polynesian vegetation at Site 1 would have consisted of grasses such as fescue (*Festuca novae zelandiae*) and silver tussocks (*Poa caespitosa*), which were extensive along the margins of large river floodplains at that time, and an array of pioneering woody shrubs such as matagouri (*Discaria toumatou*), native brooms (*Carmichaelia spp*), and vines such as *Muehlenbeckia complexa* and *Rubus squarrosus*. The outstanding feature on the rest of the plains was the presence of widespread forest vegetation dominated by the podocarps totara (*Podocarpus totara*), miro (*Pruminopitys ferruginea*), matai (*Podocarpus spicatus*) and kahikatea (*Dacrycarpus dacrydioides*) (Molloy 1969). Associated hardwoods included *Griselinia littoralis, Melicytus ramiflorus, Pittosporum* spp., *Myrsine australis, Hoheria augustifolia* and other trees and shrubs. During this time *Sophora microphylla* probably existed as a species which occupied both the open and dynamic riverbed margins and the forest environments. It is probable that *S. microphylla* colonised the younger sites (such as

Site 1) and then persisted throughout ensuing successional phases to eventually co-exist with other forest tree species. It lives quite successfully in forest environments, and evidence of this can be seen today. Wardle (1991) states that *S. microphylla* is often a member of forest remnants in the eastern South Island and a number of healthy and reproductive specimens can be found in the bush fragments remaining on Banks Peninsula. An example of these fragments can be seen in Plate 10.

Plate 10. An example of Banks Peninsula bush fragments.

Sophora prostrata was probably present on the plains at this time but only in the non-forested zones such as river margins and the banks between successive terrace systems.

Although some natural fires from lightning strikes did occur before Polynesian arrival, they became more common in Canterbury during the Polynesian era (Molloy 1969, Wardle 1991). Extensive burning led to the proliferation of shrub forests and the extension of vegetation found on the open river bed margins. The species most predominant included kanuka and manuka (*Kunzea ericoides*, *Leptospermum scoparium*), native brooms (*Carmichaelia* spp.), *Coprosma* spp. (especially *C. propinqua* and *C. rhamnoides*), matagouri (*Discaria toumatou*) and *Cassinia*

fulvida (Molloy and Ives 1972). *Hebe* spp., *Olearia* spp. and *Pseudowintera colorata* were also represented in small quantities (Ministry of Works 1983). There is little doubt that many of the forest dwelling kowhais were destroyed by these fires, but there is nothing to suggest that they did not continue to persist and regenerate on the plains after fire events. This was in contrast to the apparent failure of many of the kanuka stands, other angiosperms and large podocarps on the plains during the Polynesian era. The extensive burning is likely to have facilitated the spread and increased the density of *Sophora prostrata* on the plains due to the greater site suitability that it created, such as more open spaces.

The Europeans decimated the native plant communities on the plains (Molloy 1969, Johnston 1969, Wardle 1991). Tussock and scrub communities were burned and removed to make way for grazing land and arable pasture. This facilitated the introduction of a lot of exotic grasses including browntop (*Agrostis tenuis*), clovers (*Trifolium* spp) and sweet vernal (*Anthoxanthum odoratum*) which are the predominating vegetation at Site 1 today. Remaining forests were felled, not only to make way for pasture, but also to provide firewood and building materials for growing urban development. The activities of the Europeans were extremely destructive to all native plants found on the plains and *Sophora microphylla* and *Sophora prostrata* were no exception. The density and range of sites that *S. prostrata* occupied was reduced significantly by scrub clearing while quite a number of *S. microphylla* trees were retained as stock shelter. In areas which were not grazed, *S. microphylla* seems to display similar colonising properties to its cousins, gorse and broom, and has probably remained competitive with these adventive species because of its pioneering ability and resistance to drought (documented by Wardle 1991).

Site 2. Site 2 is on the summit and upper eastern face of the Port Hills, Banks Peninsula, and like the plains has been modified by the removal of vegetation, grazing and fire. This site is distinctly different to Site 1 in terms of climatic and soil characteristics and was not chosen especially because of this, but rather for its abundance of *S. prostrata* plants. At Site 2 many of the *S. prostrata* plants grew in tightly interlaced clumps where it was almost impossible to assess

the number of individual plants. It is estimated that there may be as many as 200 individuals at this site. Their ages vary, and this can be seen from the size of clumps although no accurate assessment of age could be made. It is approximately 2ha in size, open and bluffy with large areas of scoria rock protruding from the hillsides. Its soils are a volcanic complex with varied surfaces and material. They are described by Ministry of Works (1983) as yellow-grey and yellow brown earth intergrade with related brown granular loams at lower altitudes.

Banks Peninsula is usually considered isolated in its climatic characteristics. Although affected by the north west winds, it is more frequently influenced by the north east winds caused by air forced through Cook Strait from the predominating westerly airflows. This north or north east wind usually brings cool, moist air to Banks Peninsula.

The rainfall of Banks Peninsula has a pronounced winter maximum and due to its orographic effects, is generally higher than the rest of nearby Canterbury. Site 2, therefore, is probably slightly wetter than Site 1. However, due to its location in Dyers Pass, which is at a slightly lower altitude than the tops, the rainfall at Site 2 is documented as very similar to that of Site 1 at 800-1000mm (Ministry of Works and Development 1983). The temperature and sunshine regimes are also very similar to that of Site 1 although the higher altitude means there is usually a larger wind chill factor.

The vegetation history of Site 2 is very similar to that of Site 1, with similar vegetation types existing in forested and non-forested areas prior to Polynesian arrival. During the Polynesian era some of the original high forest was removed by fire to clear areas for garden cultivation and access. This was replaced by an array of grasses, shrubs and ferns especially silver tussock (*Poa caespitosa*), kanuka (*Kunzea ericoides*) and bracken fern (*Pteridium esculentum*). These species arose mainly from rocky enclave material within the forest zone (Johnston 1969).

Most of the native forest on the Port Hills however, was removed by the milling activities of Europeans to provide firewood and timber for growing urban development in Christchurch.

Among the cut over areas and charred stumps English grasses were surface sown, responding rapidly to the enhanced fertility of bush burns (Johnston 1969). Cocksfoot (*Dactylis glomerata*) was the most extensive of these. European arrival promoted the introduction and rapid invasion of a large number of other exotic monocot, herbaceous and woody weeds. They included fescue *Festuca rubra*, yorkshire fog (*Holcus lanatus*), catsear (*Hypochoeris radicata*), dandelions (*Taraxacum officinale*), *Crepis capillaris*, *Rumex spp* and the clovers particularly *Trifolium arvense* and *Trifolium dubium*. The introduced woody species became a dominating force in our landscape and include, common gorse and broom (*Ulex europaeus* and *Cytisus scoparius*) which are extremely competitive with native plants, not only on Banks Peninsula, but also on the plains. The proliferation of introduced grasses and weeds at Site 2 can be seen in Plate 11.

Plate 11. Grassland vegetation at Site 2. Note the large numbers of yellow flowers (*Hypochoeris crepsis*) and introduced grasses.

S. microphylla was probably extensive in the forested areas and forest margins of Banks Peninsula in pre-Polynesian times, with the more open areas on rock outcrops dominated by *Sophora prostrata*. The evidence for the close proximity of these plants to each other on Banks Peninsula is the presence of natural hybrids of the two which can be seen today at Dyers Pass

The removal of the vegetation on the Peninsula undoubtedly led to the further spread of *S. prostrata*, particularly to lower altitudes but does not seem to have promoted the growth of *Sophora microphylla* significantly. *S. microphylla* on the Peninsula seems to persist today mainly in the remaining forest fragments.

CHAPTER 3

SAMPLING TECHNIQUES USED IN EXPERIMENTATION

1. Techniques used for *Sophora microphylla*

S. microphylla produces copious quantities of seeds and the characteristics of seeds and seed set are different between individual trees. Because of this, quantities and other characteristics of seeds, on any one tree, are almost impossible to assess practically using a 100% sample. Therefore, a sampling technique had to be devised that was both random and produced a high degree of precision. Seeds are individual entities so sampling and statistical methods for discrete variables were used.

(a) Choosing the Trees

In populations of *Sophora microphylla* it was noted at an early stage in experimentation that there was a large amount of natural variability in the phenotypic characteristics of individuals. This made it difficult when choosing trees for seed sampling purposes. The differing seed age and yield characteristics between individuals meant that sample trees could not be chosen at random from the whole population. Because of this, a stratified, simple random sampling technique was used. There were two strata involved in the choice.

(i) Sample trees had to have seed of approximately the same age (within 1 week).

(ii) The sample trees had to have enough seed on them so that the removal of subsamples could be done throughout the whole course of experimentation.

After assessing which trees in the population had the required attributes, they were pooled separately from the main population. Each was given a 2 digit number and the first 5 numbers encountered using random number tables were chosen as the sample trees. Seed was taken from these trees for the entire course of experimentation.

(b) Sampling the Seed

When sampling from a population in which there is a large number of individuals, so long as the individuals within the population are thoroughly mixed, a sample from that population can be considered a completely random sample. Freese (1962) states:-

"In some populations... the individuals themselves are randomly located or can easily be made so. A batch of seed is one such population."

The assumption made here is that, regardless of location, the trees and seed used are subject to the same numbers and types of influences. In the context of seed predation for example, each seed would have exactly the same probability of being predated as the next.

(c) Sample Size

This was calculated in close conjunction with confidence limits and the following example (which is hypothetical) illustrates how the sample sizes were chosen for this project.

Suppose a population is being sampled in which some of the units have a certain attribute (eg. they have been predated), and we wish to estimate true proportion of predated seeds to within +/- 0.15 (15%). If we take a sample of 30 seeds and find that 40% (0.40) have been predated the confidence limits would be between 0.23 and 0.60. Since either limit is not within 0.15 of 0.40, 30 seeds would not be a suitable sample size. However, if a sample size of 50 was used and the proportion of predated seeds was once again found to be 0.40, the confidence limits would work out at 0.27 and 0.55; within 0.15. Therefore a sample size of 50 would be acceptable

For predation of *S. microphylla* in this particular project, the exact proportions of predated seeds were not yet known, but it was estimated that a predation rate exceeding 50% (0.50) was unlikely. Using the technique described in the above example, it was calculated that a sample of 150 seeds (30 from 5 trees) is an acceptable sample size to achieve the required level of accuracy

which has been set at +/- 0.10. The sample size of 150 seeds produces confidence limits of +/- 0.08 allowing for the event of any extraordinary results. Due to the seeds being relatively large and easy to handle, the sample size was increased to 300 seeds. This served two purposes. Firstly, it meant that the results for predation are more precise (this is important as predation is a major focus of my study). Secondly, it meant the majority of seeds required for experimental purposes could be obtained from these samples, saving on further collection. For the majority of other experimentation such as those related to germination, a sample size of 100 seeds was used with the anticipated confidence limits being within 0.15 of the observed proportion.

2. Techniques used for *Sophora prostrata*

The collection of seed for *S. prostrata* was much simpler, and less attention was given to the statistical mechanics. If, as was the case for *S. microphylla*, only the 'new season's' seed was used for experimentation there would only have been enough seed to conduct a few experiments. *S. prostrata* also produced far fewer seeds and retained them for less time after ripening. Because of these factors, as much seed as possible was collected from the plot, regardless of age, although it was ensured that all seeds were mature. Seeds used for experimentation were then taken at random from this main pool. Future workers, if they have greater time resources, may pursue experimentation with consistency of age. For nearly all experiments or experimental replicates only 30 *S. prostrata* seeds per treatment were used due to these limited resources.

3. Errors in results

Due to the large amounts of data presented in many of the graphed results the majority of standard errors have not been included in the text or as error bars, but are presented in tabulated form in Appendix 1. The standard error of the estimate (eg. estimate of the number of predated seeds) was able to be calculated for the majority of results using:

$$Sp = \sqrt{\frac{p\,(1-p)}{(n-1)}\left(1-\frac{n}{N}\right)}$$

Where p is the proporton of seeds having the specified attribute and n is the number of units observed. If the total number in the population N is extremely large relative to n (such as in this project) then the finite population correction (1-n/N) can be ignored (see Freese 1962 p62).

Where errors were obtained differently to this, the methods etc. have been identified in the text.

CHAPTER 4

IMMATURE GERMINATION AND EMBRYO MATURITY IN *Sophora microphylla*

1. General introduction

It was observed by Burrows (1991 unpbl) that some *Sophora microphylla* seeds germinated while still in their immature state. It was speculated from this that early embryo maturity may be a characteristic feature of the seed physiology of *S. microphylla*. This chapter describes a simple investigation, by germination, into whether this is the case. This particular experimentation did not include *S. prostrata* due to the limitation of seed resources outlined in Chapter 3.

2. Phases in fruit and seed maturation

Flowering and pollination of the *S. microphylla* trees at Site 1 occurs from early August to October, after which the seed pods begin to develop. Godley (1982) recognised 3 distinct phases in the development of the pods and seeds of *S. microphylla*. In the first, the seed pod elongates to full size but remains thin. The pod contains a mixture of immature, elongated seeds and fertilised ovules at this stage. In the second phase, the pods proceed to develop into their mature shape and some seeds will develop to full size. It was noticed during my experimentation that during this stage, seeds swell inside the pods to a size considerably larger than those which are fully ripe. The third phase involves the drying and subsequent release of seeds.

The seed of *S. microphylla*, at Site 1, is set in late September to early November. When first formed it is green in colour, soft and easily damaged. At around 10 weeks, the end of the first phase, seeds begin to progressively turn yellow and swell until yellowing is complete at around 14 weeks. At this stage the seeds begins to dry but are still soft, full of moisture and easily damaged. Drying of the seed and surrounding pod continues progressively until they are

approximately 20 weeks of age, when full ripening occurs. At this stage, seeds have yellowed completely and become dry with a tough impenetrable seed coat. With age the pod dries, becomes brittle and begins to disintegrate. Upon disintegration two separate wall layers can be clearly seen. The first is the inner fibrous wall which fits closely with the seeds particularly when they are immature (Godley 1982). It is often difficult to remove this inner wall from immature seeds without damaging them. When the seed matures (20 weeks) this inner wall separates itself from the seeds and they become loose inside the seed cavity. The outer wall, which includes the outer wings of the pods, completely envelopes the inner wall (Godley 1982). Unlike other legumes, there are two tough longitudinal strips of tissue either side between these wings. This makes the pod unable to separate longitudinally and release the ripe seeds. Thus the pod has to almost fully disintegrate before the seeds will drop to the ground (Godley 1982). See Plate 4 (p8) to view disintegrating seed pods. Table 2 summarises the changes in the fruit and seed during maturation, and the time frames in which they happen.

Table 2. A summary of the changes in *S. microphylla* fruit and seed during the maturation process.

State of pods and/or seeds	Age of pods (weeks)
pollination	0
elongation of pods to full size	0-4
development of pods into mature shape, seeds become full size	4-10
yellowing of seeds	10-14
drying of pods and seeds	14-20
release of seeds from disintegrating pods on the tree	30+

3. Methods

While *S. microphylla* seeds were in their immature state 300 were collected, at random, every 2 weeks to investigate predation. From these 300 seeds, 100 healthy seeds (4 replicates of 25) were taken and used for experiments in this chapter. Experimentation commenced at the time the seeds were able to be practically handled. This was approximately 6 weeks of age (from fruit set). The age of the seeds was determined by estimating the amount of time elapsed between fruit set and collection of the seed.

The short time period between the collection of replicates, 2 weeks, was considered important for locating the embryo maturity time as accurately as possible. Extreme care had to be taken to ensure seeds were damaged as little as possible, as the more they were damaged, the more susceptible they became to microbial attack, particularly from saprophytic fungi.

Each replicate of 25 seeds was then sown on germination pads, in sterilised 9cm petri dishes. To moisten the germination pads, 15ml of sterilised water was used. Sterilised water was used as an extra precaution against microbial infestation of the seeds. Initially, the amount of water each replicate was given was exactly the same (15ml) to try to maintain uniformity. Slightly differing imbibition characteristics between seeds after initial sowing meant that each replicate may have used slightly differing amounts of water. Because of this, after the initial sowing date the amount of water in each petri dish received was only kept as uniform as practically possible.

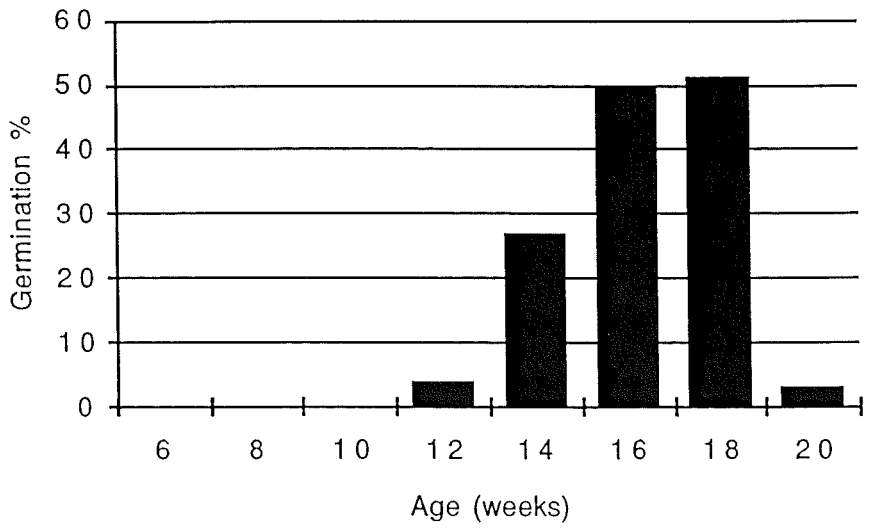
The replicates were then placed in a glasshouse exposed to ordinary sunlight. The glasshouse maintained temperature at a uniform 20°C and uniform humidity. The replicates were then monitored at three day intervals for the numbers germinated, if any.

4. Results

Along with the visible ripening, the impermeability of *S. microphylla* seeds was considered a good 'yardstick' in assessment of full maturity. When seeds failed to imbibe they were considered to be mature, as impermeability of the testa was thought to be one of the last developments before maturation. At 18 weeks 51% of seeds germinated without scarification. Within 2 weeks, this figure had decreased from 51% to 3%, indicating a rapid change in the status of the seed coat in the final weeks before seed maturation. Changes in the status of the seed coat in the last few weeks before maturity has been documented as occurring in legumes for some time. For example Helgeson (1932) found that similar changes occur in *Melilotus alba.*

No germination was noted until the seeds were 12 weeks of age. Figure 3 below shows total percentage germination of immature seeds over time.

Figure 3. Total germination percentages for immature *Sophora microphylla* seeds by age (wks). **(Errors tabulated in Appendix 1 p127)**



The results show that, in accordance with the the definition of maturity (ie. when seeds are yellow, dry and fail to imbibe without scarification), *S. microphylla* seeds do germinate in their immature state. The maturity of the majority of embryos seems to occur at between 14 and 16 weeks of age, although there is a definite range of maturity times, with some seeds maturing as early as 10 to 12 weeks. The control, for this experiment was the germination success of mature seeds in optimum conditions (20^{o}C, abundant moisture). Replicates under these conditions seldom exceeded a 65% success rate.

Although there is approximately a 15% difference between the figure for seeds germinating at 18 weeks (51%) and full maturity, the high amount of microbial activity and its ensuing destruction of some embryos at this time could account for this.

Immature seeds seemed to attract microbial activity during these experiments, especially when damaged. There was a significant reduction in this activity as seeds approached maturity. In the latter phases of experimentation, 16 and 18 weeks, the action of microbes was curbed to a large extent by the surface sterilisation of seeds with a 1% solution of household bleach. Seeds which were younger than 16 weeks were not surface sterilised, as it was feared the bleach would destroy the embryos of the young, fragile seeds.

Another important observation made during these experiments was the presence of a high quantity of 'yellow green' leachate which exuded, particularly from very young green seeds, once they had been placed in water. The exact composition of the leachate is not known but it is thought to be a culmination of nitrogenous and colloidal compounds assimilated into the seed coat during development.

5. Discussion

The study of the maturity of seeds is not a new one, but much of the work has tended towards the physiological and biochemical changes associated with seed maturity, rather than its ecological significance. The study of the maturity of seeds with hard and often impenetrable seed coats has received some attention, but is still not well understood.

This simple experiment has shown only that it is possible for *S. microphylla* seeds to germinate when they are immature, but this early germination is not considered to be the normal behaviour of the plant. The ecological significance of a plant being able to mature its embryo early is that seeds removed from the plant prematurely, due to adverse conditions or situations, may still have the ability to germinate anyway, thereby maintaining its reproductive capability. *S. microphylla* normally maintains its seeds on the tree until maturity and often well beyond, but if a large quantity of immature seed were to be removed from a population, it is possible that some may germinate.

If fruit was removed from the tree prematurely, the seeds would not be released easily, and consequently germination would be low anyway. The pods in which the seeds are housed, are green and full of moisture in their immature state. They are malleable, flexible and connected to the interior wall of the seed pod, making their release difficult. The only way that seeds could be released is by microbial decomposition of the pod. Unfortunately, the seeds themselves are very prone to microbial decomposition, particularly after damage or bruising which is likely to occur in the natural environment. Therefore, destruction of the embryo before germination takes place is likely to occur. Once released the seeds would also have to contend with the microbial activity in the surrounding environment, particularly the soil, and have to overcome this before germination could occur.

Despite this, it is interesting to observe that seeds which are immature will imbibe and germinate. The imbibition of seeds was observed from a very early age (8 wks). These seeds were not scarified and there is a period of time, between approximately 16 and 20 weeks of age, when germination percentages of immature, unscarified seed was approaching that of mature, scarified seed.

It was only in the final 2 weeks of immaturity (18-20 wks) that the seed coats of *S. microphylla* became impermeable to water. The fact that germination was achieved easily before maturity of the seed coat tends to suggest that the physical seed coat is the only mechanism preventing the penetration of water in mature seed.

Many workers support the idea that for the majority of leguminous seeds like *S. microphylla*, water impermeability is the major, if not the only, factor controlling dormancy. Bewley and Black (1982) state that the part of the seed coat responsible for the lack of water penetration in legumes is a waxy cuticle layer which surrounds the outside of mature seeds. Helgeson, as far back as 1932, found that the seed coats of *Melilotus alba* (sweet clover) changed from the water permeable state to the water impermeable state as a final stage in the maturation process. This was attributed to an irreversible change in colloidal activity inside the seed coats during dehydration.

With *S. microphylla*, the scratching or even removal of the waxy cuticle will not necessarily promote germination. It was found in experimentation with mature seed, that the integrity of the seed coat had to be entirely violated to guarantee germination. This suggests that there is perhaps a combination of both a waxy cuticle and colloidal material within the seed coat preventing water entry. Immature seeds used in these experiments had not yet developed an impermeable cuticle and the majority of soluble leachates (the yellow green leachate) seemed to be drawn out during the experimental process. This may have allowed imbibition to take place, and germination to occur in seeds whose embryo was mature enough. It is also entirely possible that the material from which the seed coat itself is constructed may be responsible for water impermeability. Resilient organic material like this can be found elsewhere in nature, for example in pollen grains.

This project has also not considered the condition of the micropyle. It is feasible that the transition from the impermeable to the permeable state may be attributable to a change in its status after exposure to microbial activity.

CHAPTER 5

SEED PREDATION IN *Sophora microphylla*

1. General introduction

Sophora microphylla seeds are large compared to a lot of other leguminous species, which in general tend to be small (Bradbeer 1988). Because of this they pose a good potential food source to predatory organisms. The first aspect of predation which had to be addressed was identification of organisms predating *Sophora microphylla* seed. This involved basic research, mainly observation, on the interactions that the plant had with potential predators in its environment.

The potential predators fell into three main categories. The first was larger predators such as birds and animals. The animals thought to possess the greatest threat were rodents, but possums, rabbits and domestic stock were also considered potential threats. The second was insects, particularly larval stages which are well known as seed predators and the third was microbial organisms.

The plant and its seed contains toxic phenol's (Connor 1982). Therefore, as expected, the fruit and seed posed no attraction to birds or animals. Even birds feeding on the nectar of the flowers were rare. There were a lot of common birds at the site such as blackbirds, finches (including sparrows) and thrushes but many of the known pollinating birds such as silvereyes, bellbirds and tuis were rarely seen. At no time were any larger predators seen to eat the fruit or seed of *Sophora microphylla*, nor was there any physical evidence such as bite marks or caches of seed to prove that they were using the seeds as a food source.

Microbial activity was very apparent on immature seeds but this became less pronounced as they approached maturity. Mature seeds were only prone to microbial breakdown when they had been scarified or damaged. In fact, mature seeds, so long as their seed coats remain intact, seem to

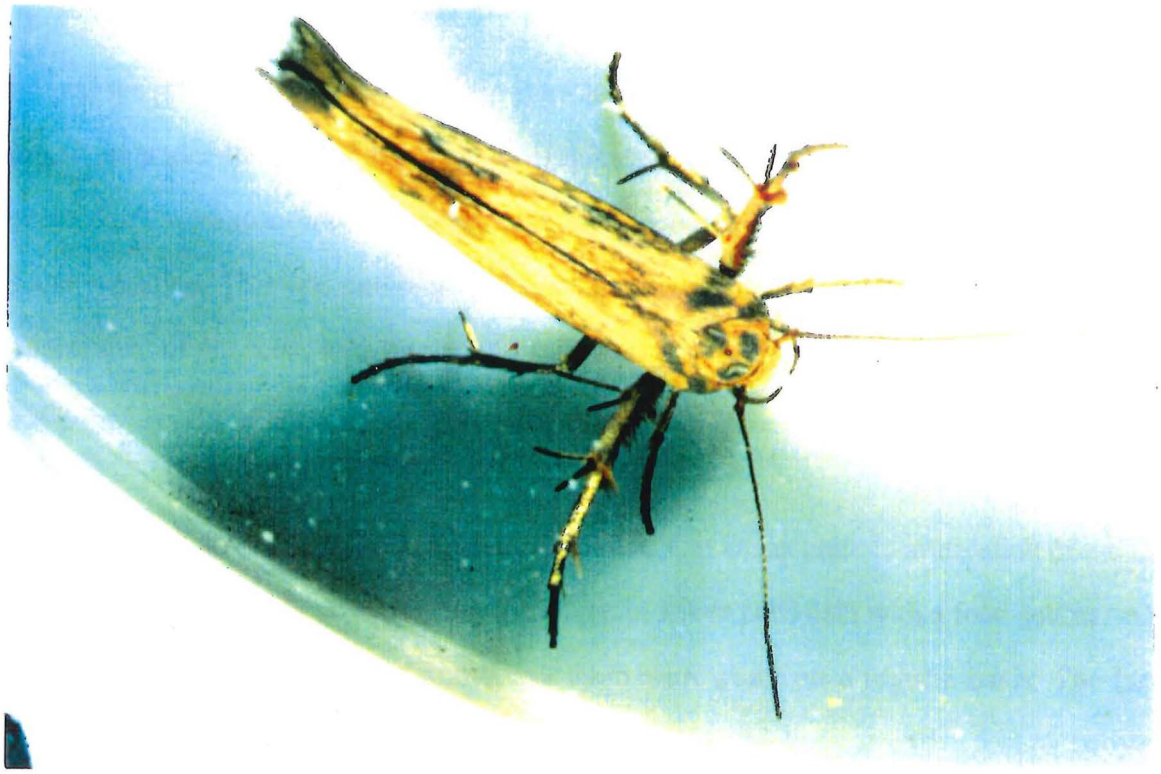
possess a natural resistance to the effects of microbial activity (Greenfield 1992 unpubl). It seems therefore, that microbial activity is probably not directly responsible for the predation of seeds.

However, a seed predator in the form of a moth larvae was found. It proved to be a very varacious predator of *S. microphylla* seeds and was absolutely detrimental, destroying them completely. This was the larvae of the moth *Stathmopoda aposema* (Oecophoridae: Stathmopodinae). This moth larvae also predates the seeds of *S. prostrata*, but seems to do so to a lesser extent. It was the only organism seen to cause large scale destruction in *S. microphylla* seed populations. This predation study centres mainly on the effects of this organism. Plates 12A and 12B show the *S. aposema* larvae and adult moth respectively at about 10x magnification.

Plates 12A and 12B. The (A) larvae and (B) adult of the Kowhai seed moth *Stathmopoda aposema.*

(A)

(B)



2. Methods

A random sample of 300 *S. microphylla* seeds was taken every two weeks until the seeds had reached maturity. Once the seeds had matured samples were taken on a monthly basis. As for experimentation on embryo maturity, the time between samples was small initially because an accurate indication was needed of when predation began. The sample was comprised of 60 seeds taken from different parts of the canopies of each of the 5 sample trees.

The whole fruit were then brought back to the laboratory where the seeds were removed from their pods and studied for predation. Several kinds of information were recorded: the total amount of predated seeds in the sample, the number of predated seeds in each pod and the total number of seeds in each pod. This was to assess whether there was any selectiveness by *Stathmopoda aposema* towards pods with larger numbers of seeds. Finally the numbers of larvae, pupae and

adults were recorded to attempt to try and establish some basic information on life history of the moth (when pupation takes place and seasonal differences in larval adult and pupal stages).

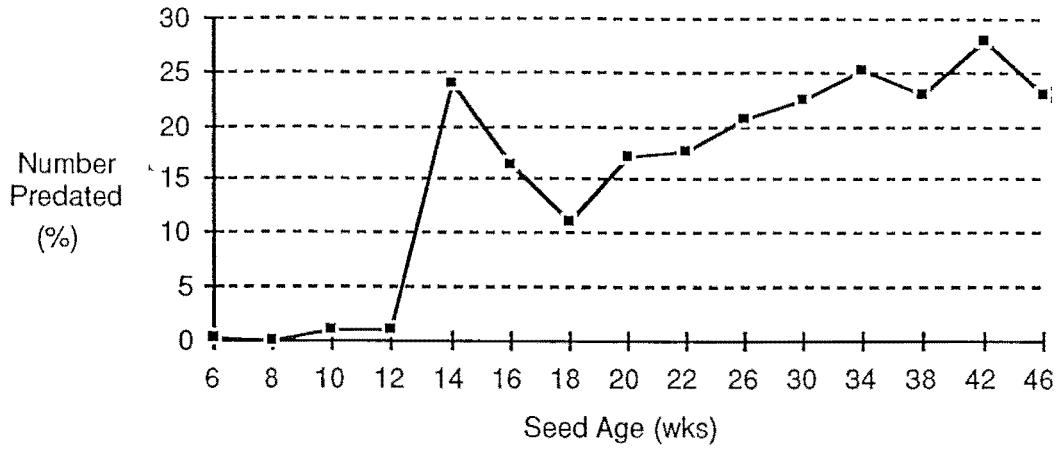
Individual seeds and seed pods were examined at various stages during the predation process to try to gain an understanding of the methods of infestation and feeding employed by *Stathmopoda aposema*. Pods were examined externally and, after careful longitudinal dissection, internally for eggs and predation. This was done under a binocular dissecting microscope. Notes were made on the appearance of the seeds and the feeding methods of *S. aposema* during the larval stage of its life cycle.

Some larvae were grown through to adulthood in order that an accurate identification of the moth could be made. This was done by placing the larvae in a 9cm petri dish with a food source, in this case partially predated seeds, and sprinkling them with water on a regular basis. The larvae pupated quite successfully under these conditions.

3. Results

(a) Seed Predation Predated seeds were found very early with the first predated seed and larvae found when the seed was only 6 weeks of age. Despite this, predation figures tended to be very small until the seeds were 14 weeks of age. At this time there was a dramatic increase in the number of larvae, and predated seeds. Figure 4 shows the percentage of predated seeds found at each sample age.

Figure 4. The number of predated *S. microphylla* seeds with respect to seed age. The seed ages correspond to the times at which samples were collected. **(Errors tabulated in Appendix 1 p127)**



The large jump in the number of predated seeds between 12 and 14 weeks of age (January) was thought to be the result of mass hatching of the *Stathmopoda aposema* larvae at that time. It was expected that if predation occurred in approximately the same density throughout the whole seed population, the number of predated seeds should increase steadily over time. However, somewhat of an anomaly occurred in the following sample (16 weeks) because an unexpected reduction in the percentage of predated seeds (by over 50%) was observed. Although the reason for this is not clearly understood, it is likely that the sample taken at 14 weeks had, by chance, an unusually high proportion of predated seeds. This does not necessarily conflict with the fact that there was definitely a major increase in the numbers of predated seeds and *Stathmopoda aposema* larvae at that time. It may be, that the fall in their numbers was caused by some sort of predation of the larvae. However, this is unlikely, considering how well they are protected they are inside the hard walls of the seed pods and seeds themselves.

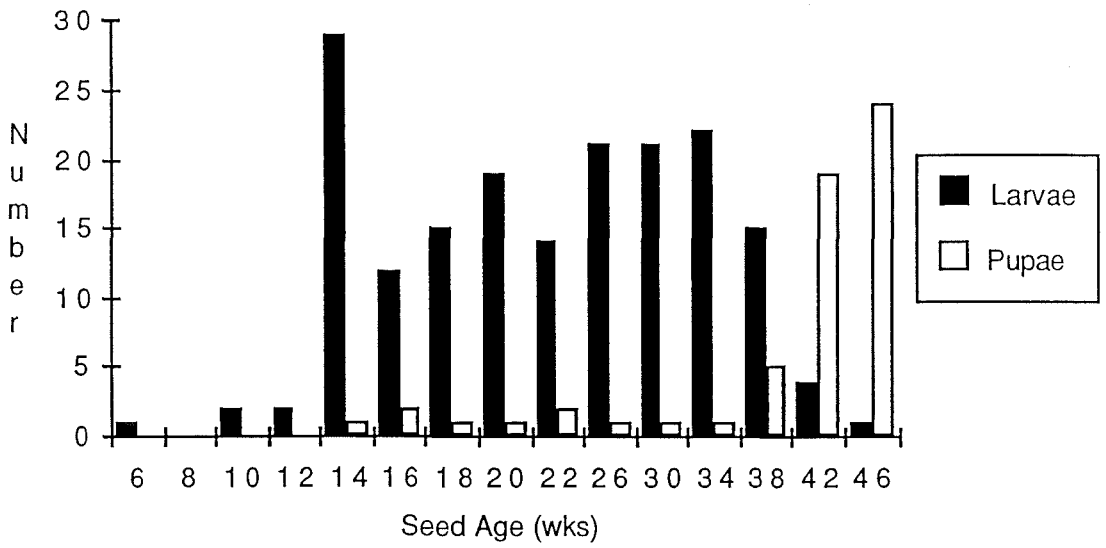
Because it was assumed that the number of predated seeds in each sample was representative of the magnitude of predation in the whole population, the total percentage loss of seeds in the year

is estimated to be when the sample predation is greatest. This occurs at 34 weeks, when the level of predation reaches 28%. It must be noted however, that this figure is based on seed predation data over only one year, and the total number of seeds predated may change from year to year depending on the timing of flowering and adult moth emergence. The density of *Stathmopoda aposema* may also fluctuate, depending on external influences such as the duration of optimum environmental conditions for completion of its life cycle, or parts thereof and influences of predators and disease.

As was anticipated, there was a general increasing trend seen after 16 weeks. One larvae would often eat 2-3 seeds per pod in one year, but in an ordered fashion, one after the other. The steady increase in predated seeds throughout the year is thought to be a reflection of this.

The trends in the numbers of larvae and pupae of *Stathmopoda aposema* over the sampling period are shown in Figure 5. Because there is no upper limit in the amount of larvae and pupae that could be found, errors had to be calculated on the mean number of each recorded throughout the year. The mean number of larvae found over the year was 11.35 per 300 seeds. The error on this was 0.1111 larvae per 300 seeds (ie. mean number of larvae found is 11.35 +/- 0.1111). Likewise the mean number of pupae found was 3.87 per 300 seeds and the error on this figure is 0.0944 pupae per 300 seeds (3.87 +/- 0.0944). These errors were calculated using the formula for count variables (Freese 1962 p68-70).

Figure 5. The number of *Stathmopoda aposema* larvae and pupae found in *Sophora microphylla* seed pods by age.



There are two main points which can be made about the trends in Figure 5. Firstly, and not unexpectedly, the trend in the larvae numbers closely mirrors the trend in the number of predated seeds shown in Figure 4, with a similar pronounced increase at 14 weeks. This supports the finding that *S. aposema* larvae are the only major predator of the seeds. Secondly, and perhaps more importantly, is the distinctly rapid drop in the number of larvae and corresponding increase in the number of pupae as the next seed season approaches. Pupation begins to increase when the seeds are approximately 38 weeks of age; in the following August after seed set. This confirmed early suspicions that the larvae of *S. aposema* overwinter in the seed pods, and emerge to reinfest the new seed crop in spring.

(b) Mechanism of infestation When examining the method of infestation, it was thought that the most likely way which *Stathmopoda aposema* could infest the seed pods was by ovipositing egg(s) onto the outer surface of the seed pod. After this they proceed to hatch and the larvae chews through the outer wall and into the seed cavity.

Scrupulous inspection of hundreds of pods at a range of different maturities was conducted to try and isolate eggs or entry holes. This was done both microscopically and with the bare eye initially concentrating on the exterior of the seed pod.

Although no eggs were found, tiny holes were discovered through the wall of pods of all ages These holes were too small and appeared too soon in the fruiting season to be exit holes fo emerging adults which is what they were initially thought to be. Plate 13 shows one of thes holes at about 20x magnification. Although never observed, it is thought that these holes wer made by the larvae chewing their way into the seed cavities after oviposition of eggs onto th exterior of the seed pod.

Plate 13. Suspected entrance hole of *Stathmopoda aposema* larvae into a *S. microphylla* seed pod.

The exact age of the seed pods at which the adult lays its eggs is unknown, but it is thought t occur when the fruit is very young, around 3-4 weeks of age. This is assuming that the adul emerge, mate and reinfest immediately. The 3-4 week age of the seeds coincides with th emergence of the majority of adults from previous season's seed pods in mid to late October. Th time of adult emergence was discovered by accident when seed was collected for predatic assessment. Some seed pods were collected on 15 October and examined for predation th following day. In that short period of time, (approximately 16 hours) 8 adult moths had emerge

and been trapped inside the plastic bag. The emergence of adult moths had not been seen at all prior to this date. The absence of eggs from pods could have simply been because the majority if not all eggs had hatched by the time pod inspections were carried out. The fact that it was not known what the eggs looked like and their exceedingly small size probably did not aid the search also.

(c) Method of predation There is no doubt that the predation of *S. microphylla* seeds by *S. aposema* was absolutely detrimental. Once predation of a seed had taken place it had no chance of germination as the embryo and cotyledons were completely destroyed.

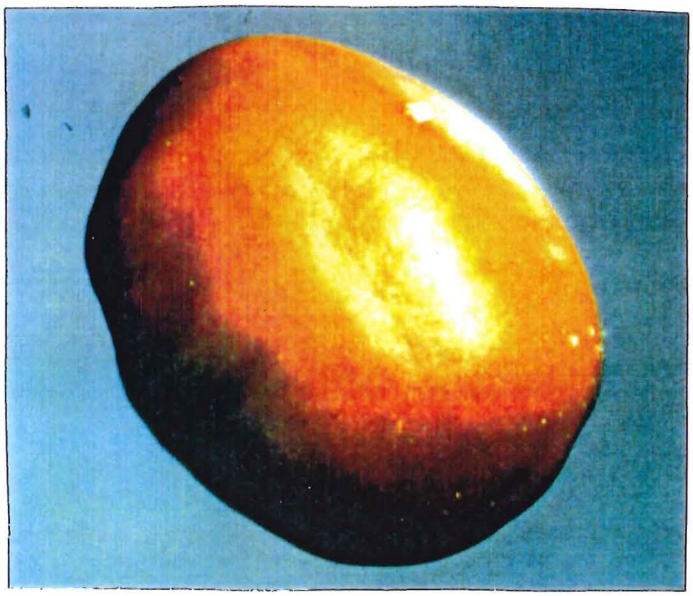
When hatched the larvae of *Stathmopoda aposema* chews a small hole in the testa of the seed and then proceeds to devour its inner contents, particularly the cotyledons, but also the embryo. At first, due to the tough unpalatable nature of the seed coat, it was suspected that the larvae entered through the micropyle but this was later discovered not to be the case. The larvae seemed to prefer no particular entry point into the seed and appeared to enter the seed at the point of first contact. One seed was only ever predated by one larvae, and as the larvae grew the hole in the seed testa was made larger to facilitate exit from the seed if necessary. The seed coat itself was seldom eaten and fragments of uningested seed coat could be clearly seen under a microscope. Often, but not always, these fragments, along with faeces, were mixed with a silk extruded from the posterior of the larvae to fill the hole in the seed coat making a completely enclosed feeding environment.

A larvae would not necessarily eat only one seed in the course of its life, but would often consume one or more adjacent seeds before pupation. Neighbouring seeds were reached by chewing holes between seed cavities. These seeds were always adjacent to the initially predated seed and the larvae seemed to show no preference for particular seeds. After predation, seeds appeared to be very prone to fungal attack. Often larger larvae were found in seeds almost totally decomposed by fungi. At no time however, would fungi spread to other healthy seeds in the pod

and perfectly healthy seeds were found in the same pods as those under fungal attack. Plates 14 (A-F) give a progressive perspective of the predation taking place.

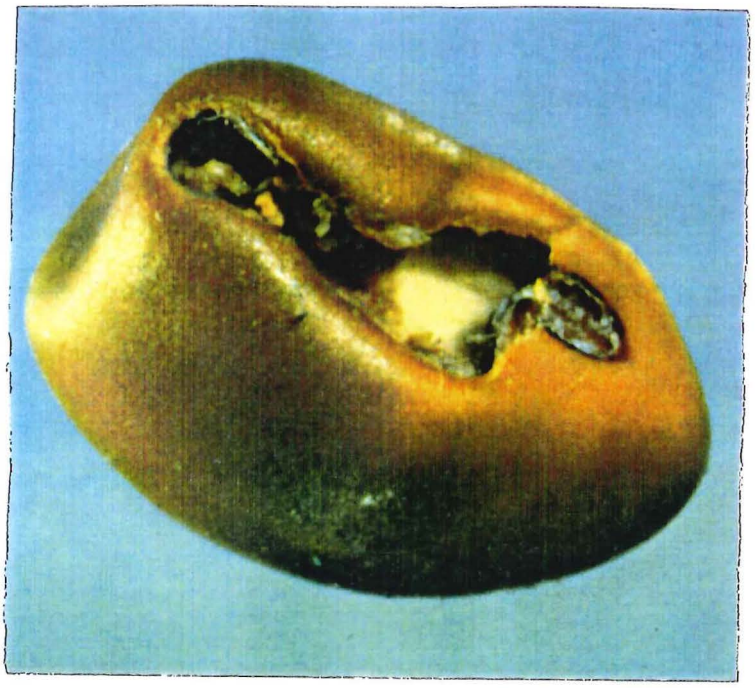
Plates 14 (A-F). Sequence of Sophora *microphylla* seed predation by *Stathmopoda aposema* larvae.

(A) A healthy *Sophora microphylla* seed.

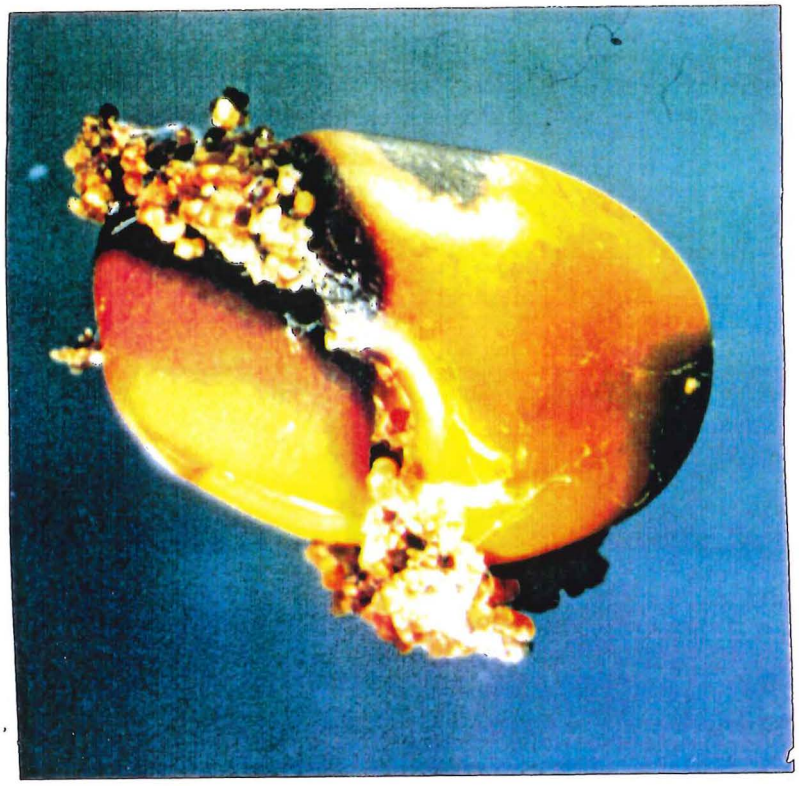


(B) *Stathmopoda aposema* has chewed through the seed coat.

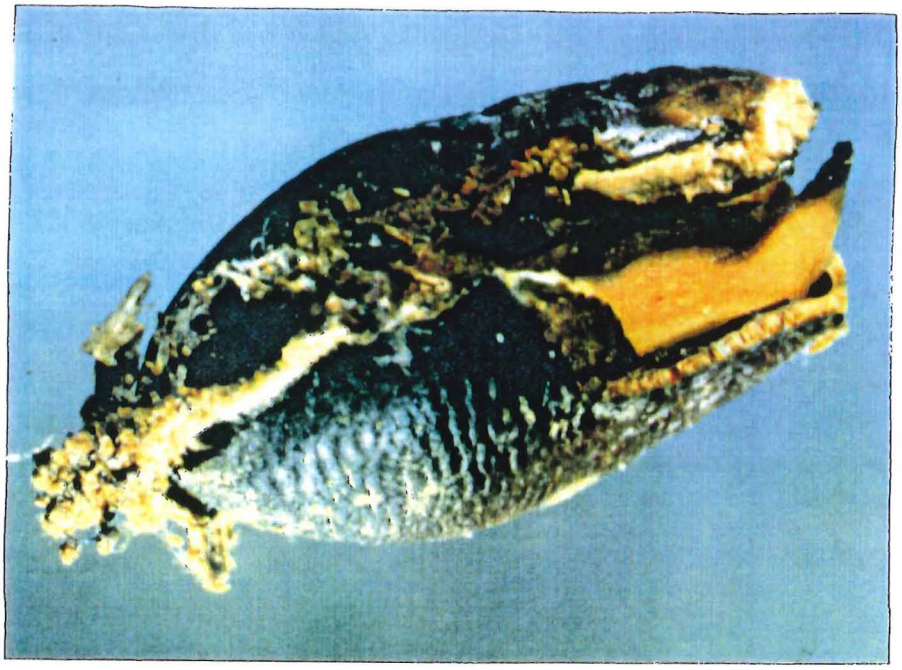
(C) *Stathmopoda aposema* starts eating the interior of seed and enlarges the hole in the seed coat as it does so.



(D) *Stathmopoda aposema* closes the hole in the seed coat with the silk/seed coat/faeces mix.



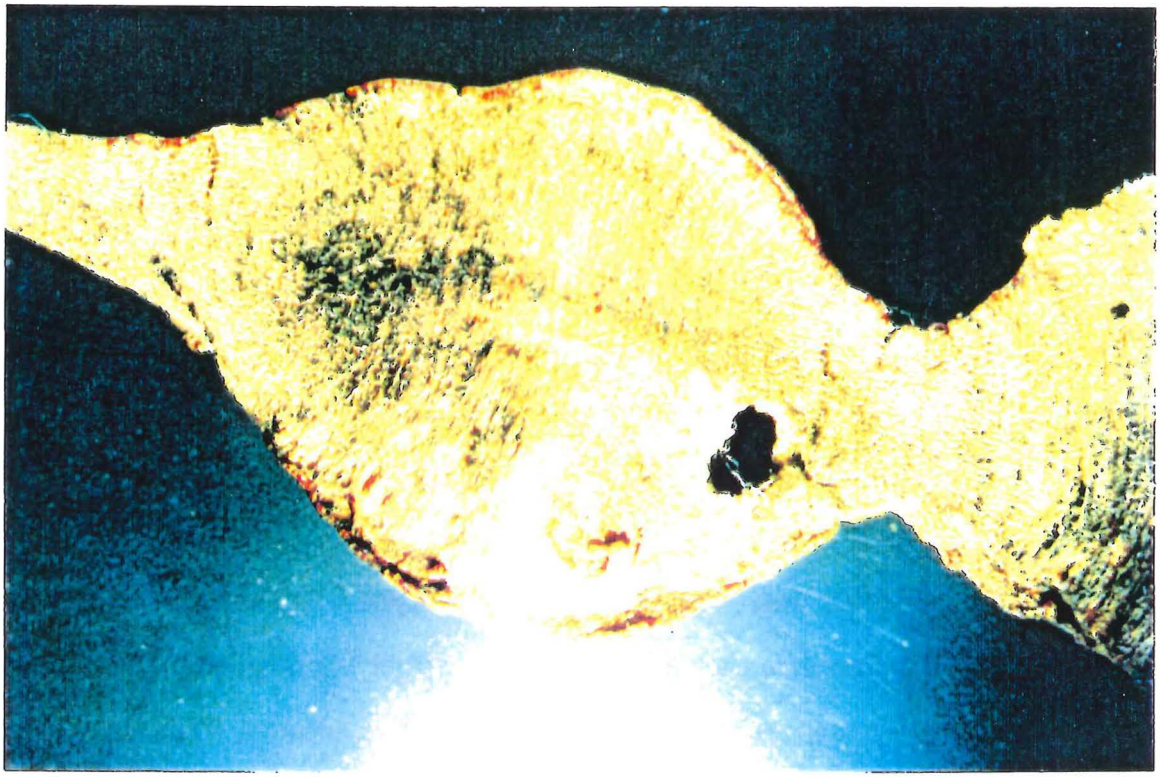
(E) The seed dies and begins to decompose.



(F) Predation of the seed is complete. At this stage *Stathmopoda aposema* either pupates or proceeds to devour the neighbouring seed in the pod.

Upon pupation the larvae uses either the hollowed out seed coat it created, or the seed cavity tc pupate. It wraps itself in a shallow, almost transparent cocoon made of silk, and then emerges a: an adult in approximately 3 weeks. An emergence hole can be seen in Plate 15.

Plate 15. An emergence hole in the seed pod of *Sophora microphylla* for the adult moth of *Stathmopod aposema.*



The imminent disintegration of *S. microphylla* pods and subsequent release of the seeds can b prevented by pupating *S. aposema.* This is due to the silk it attaches to the wall of the seed po inside the seed cavity. It is thought that this is an aid to ensure development is completed. Thi has been observed in the field by Auld (1983), for insect pests of *Araecerus* sp. and in *Dillwyni retorta* var *retorta.* It is extremely unlikely that *S. aposema* could survive on individual disperse seeds and they rely on intact and sound seed pods for their successful development.

Although there seemed to be a preference for 3 and 4 seeded pods in the predation patter *Stathmopoda aposema* probably has negligible preference. The reason for this is that the majorit

of *S. microphylla* pods contain 3-4 seeds and therefore it is logical that the frequency of predation should be greatest in these pods.

4. Discussion

The only information of substance on *Stathmopoda aposema* has been provided by Hudson (1928). He describes the moth as a large species occurring at Auckland, Wellington, Dunedin and Lake Wakatipu. It is apparently very local. The expansion of the wings are almost 3/4 of an inch (20mm). The forewings are pale brown ochreous; the costa is breadly edged with dull brownish grey from the base to 3/4. There is a longitudinal brown streak in the disc almost from the base to the apex. The dorsum is broadly edged with brownish grey. The hind wings are dark grey. The cilia of all the wings are dark greyish ochreous. The perfect insect occurs in October, November and December, frequents forests and has been documented on other plant species such as the narrow leaved lawyer vine (*Rubus cissoides*) (Hudson 1928).

Other studies have indicated that many angiosperms lose their undispersed seeds to insect herbivores (eg. Crawley 1989). In fact, from other work done on leguminous species, it appears they are particularly prone to seed predation. Auld (1983) for example, in his study of native legumes in South Eastern Australia, found that all 28 species (11 genera) under investigation suffered some form of seed predation. Of these 28 species, 22 (8 genera) had seed crop losses greater than 10%.

Auld (1983) also documents some parallels between the predation of leguminous seeds in his study and this study of *Sophora* seed predation. For example, weevil eggs hatch and the larvae proceeded to burrow into the seed and feed on its contents. Only one larvae can develop in any one seed and it will often consume one or more adjacent seeds before pupation.

It was also interesting to find that there was only one primary predator, another aspect which is not uncommon among legumes. Auld (1983) for example found that many of the species he studied were primarily attacked by species from only one insect genus.

There are some significant ecological implications for the predation of *Sophora microphylla* seed. The origin of *Stathmopoda aposema* in New Zealand is not known, but it is likely that it has been present for a long time. In Canterbury at least, the moth does not seem to be localised, or prolific in any one lowland area, although there seems to be a distinct drop in its presence with altitude. This may explain why *S. aposema* was not very common in the *Sophora prostrata* seed populations on the Port Hills.

S. microphylla individuals, in general, seem to have evolved to produce huge amounts of seed and fruit (pods) which contain large numbers of seed. In insect predation of whole fruits, some plants have evolved to produce large fruit crops, as a large fruit crop will often suffer a lower loss rate than a small one, presumably because insect abundance and fruit density are not closely coupled or are coupled with a substantial time lag (Crawley 1989). For example, the affect of a particular species of seed predator on a plant population in one fruiting season will be influenced by the dispersal ability of the seed predator and the timing of fruit development and maturation in other populations of plants also infested by this same species (Auld 1983). It is feasible to suggest therefore, that *S. microphylla* has evolved with *S. aposema*, and in doing so now produces copious quantities of seed in an attempt to satiate its predatory activities and ensure enough viable seed is produced to recruit new individuals into the population. Louda (1982) states that seed predation will be significant in plant dynamics if and when seed supply limits recruitment to below the sustainable density. However, a complication in all this is the considerable longevity of *S. microphylla* plants. Over many seasons individuals undergo good and bad flowering efforts and undoubtedly large and small losses due to predation (depending on the status of *S. aposema* in that particular year). Therefore, there will be some years, for example when flowering is poor and predation is large that predation may be devastating to seed populations on some individuals.

The probability of death of any individual seed declines as the number of seeds per fruit increases (Crawley 1989), which may explain the significant number of *S. microphylla* pods with large numbers of seed. The high amounts of natural variability seen in the flowering times of *S. microphylla* individuals may also be a mechanism which has evolved to confound the egg laying activities of *S. aposema* in an attempt to preserve seeds from predation.

It is not certain whether *S. microphylla* is a mast seeder although the numbers of flowers and seed set each year do vary. If it is a mast seeder there would be obvious benefits such as predator satiation. Kjellsson (1985) states that a plant involved in predator satiation (the strategy of which was suggested by Janzen 1971), should have a large seed crop, fast ripening and short dispersal periods. *S. microphylla* possesses the first two of these traits but appears to fall short on the third, retaining much of its seed on the trees for long periods of time. This may be the reason that it builds up such extensive soil seed banks and has evolved such an efficient dormancy mechanism in the tough impenetrable testa. The build up of enormous quantities of dormant, viable seed in the soil would counteract seed losses due to reinfestation of old seasons seed. Despite this, there seemed to be minimal reinfestation of older seed pods. Table 3 shows predation figures for a study of 600 previous season's seeds, done in January to see if there was any evidence of reinfestation. The methods employed for the study were exactly the same as those used for new season's seeds. The ages of the seeds was not known but it is unlikely trees have retained this seed for more than 12-18 months.

Table 3. Predation characteristics of previous seasons *S. microphylla* seed.

Number of Seeds Examined	Number Predated (%)	Number of larvae	Number of Pupae
600	26.2 (+/- 1.9)	0	24

The reinfestation of previous seasons seed would be indicated by the presence of larvae in the seed pods at this time of the year. No larvae were found, only pupating moths, suggesting that reinfestation of these seeds is extremely low if not non-existent. As a result of this there seems to be no significant risk of further seed loss after the first seasons seed attack if *S. microphylla* retains its seeds for long periods of time.

Seed predation is something which *S. microphylla* seems to be dealing with successfully. There are still copious quantities of viable seed produced every year most of which finds its way into the soil seed banks.

CHAPTER 6

SEED FALL, SEED DISPERSAL AND SEED BANKS

1. General introduction

This chapter describes the fate of seeds after they have ripened. It briefly addresses the seed fall characteristics of each species and what happens to the seeds after they have fallen, in terms of dispersal and recruitment into seed banks. *S. microphylla* and *S. prostrata* are dealt with separately in each of this Chapter's three sections: seed fall, seed dispersal and seed banks.

2. Seed Fall

(a) *Sophora microphylla*

Introduction

S. microphylla retains some seed on its trees for considerable amounts of time (suspected to be about 2 years maximum) leading to a build up of 'tree seed banks'. The amount of seed retained over time, and the magnitude of these seed banks is not known. The apparent reliance on wind to dislodge seeds from the tree (Godley 1982) would suggest that the magnitude of seed fall and recruitment of seeds into both soil and tree seed banks would vary from site to site and also throughout the year, and between years. It became important, therefore, to assess characteristics of seed fall of *S. microphylla* experimentally in order to determine the amounts of seed lost and retained by the tree in a year, and to examine the mechanisms by which seed are released from the fruit. Unfortunately, data for differences in these characteristics between years could not be attained due to time limitations.

Methods

The only way which the timing and magnitude of seed fall could be assessed accurately in *S. microphylla* was to mark some new seasons seed and to monitor its loss over time. Because it was impossible to mark individual seeds within the pods, the individual pods themselves had to be marked. Hence, 150 pods, 30 from each of the 5 sample trees were marked. Pods were marked in different parts of the canopy to ensure that there was no bias towards any one wind direction, and the positions of the seed pods was noted. They were marked with bright red nail polish, which retains its colour and durability for long periods of time in the open. Pods were re-marked if they became faded.

Anecdotal evidence suggests that the seed pods begin to fall in April. This corresponds with the strong northwest winds at this time. Therefore pods were marked at the end of March (30th), and their accumulated loss recorded at the end of each month, until the following season's seed had been set (estimated to be October). The average number of seeds per pod in *S. microphylla*, although varying slightly from tree to tree, is about 4 (personal obs). Therefore, in the interests of maintaining uniformity and simplicity all seed pods marked contained 4 seeds.

Results

The percentage of seed pods (of the total marked) that each tree had lost between April and October is given in Table 4.

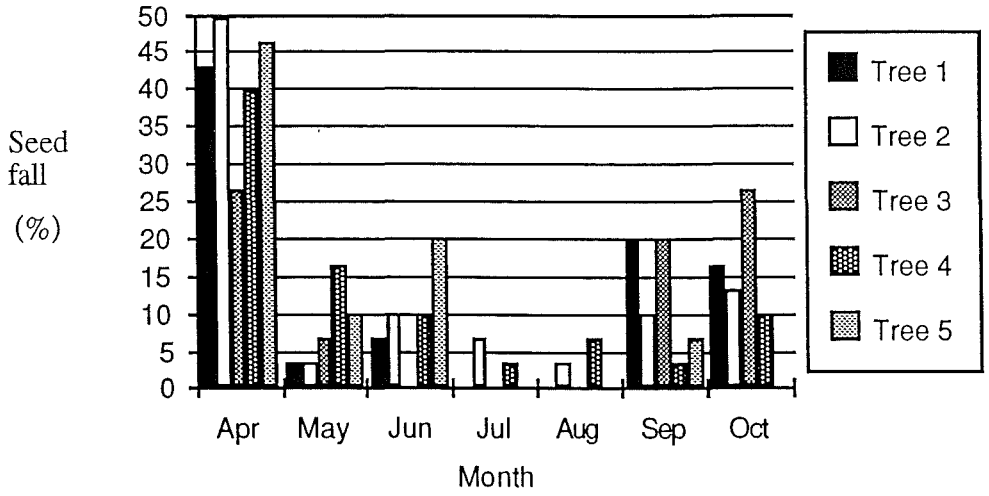
Table 4. Pod fall for 5 *S. microphylla* trees April 1992- October 1992. **(Errors tabulated in Appendix 1 p128).**

Tree 1	Tree 2	Tree 3	Tree 4	Tree 5
89.1	96.7	79.2	89.1	82.5

It can be seen that in the course of this particular year, *S. microphylla* has lost the majority of its new season's seeds, and there are only 2 trees out of the 5 which have retained significantly more than 10% of its seed into the next seeding season (post October). The average seed retention over the 5 trees is only 12.7%. However, 12.7% could account for a substantial number of seeds on some individual trees. For example, on one individual, whose total seed production was estimated at 76 000, 12.7% would account for nearly 10 000 seeds.

The timing of seed fall seems to be closely correlated with times of the year in which strong winds are prolific. Figure 6 shows the proportion of seeds lost each month for the 5 trees.

Figure 6. Pod fall per month for 5 *S. microphylla* trees, April 1992-October 1992. **(Errors tabulated in Appendix 1 p128)**



The substantial seedfall in April for all 5 trees indicates that *S. microphylla* loses much of its seed in the early autumn. This is followed by a distinct drop, almost to nil during winter, only to increase again in the spring. The lower seed fall in the spring compared with the autumn may be because the seed volumes on the trees are less by this time. The predominance of seed fall in autumn and spring co-incides with the equinoxes and consequent predominance of strong north westerly winds. The frequency of northwest winds appeared to be greatest at the times when seed

fall was greatest (personal obs). Before experimentation began, it was anticipated that this pattern of seed fall would be observed in *S. microphylla*. At calmer sites there may be much less seedfall than was recorded here.

(b) *Sophora prostrata*

Because of the straight forward nature of the seed fall characteristics in *S. prostrata*, most of the information on seed fall has been attained through observation rather than experimentation.

S. prostrata displays similar seed fall characteristics to *S. microphylla*, although its seed fall patterns are more easily explained. Unlike *S. microphylla*, *S. prostrata* tends to lose the majority of its seed immediately after maturity and retains little on the plant. This occurs mainly in the autumn (from March through to June). The much quicker release of the *S. prostrata* pods is probably a factor of the lighter construction of its pedicel and ease of removal in relatively light winds. The environment of *S. prostrata* used for this study (summit of the Port Hills) is extremely exposed and probably subject to more frequent high winds. This may explain the short seed retention time observed. However, the *S. prostrata* individual at Site 1 (*S. microphylla* site) showed almost identical characteristics, suggesting that a long flowering season and quick release of seeds is a characteristic of the species.

3. Seed Dispersal

(a) *Sophora microphylla*

It was found that *S. microphylla* was very versatile in its use of natural dispersal mechanisms. As mentioned earlier, the size of the seed, toxicity and toughness of its seed coat, and apparent impalatable nature of the fruit, meant that dispersal by animals and birds was unlikely. From observation it was found that *S. microphylla* uses three main mechanisms for the dispersal of its seed.

(a) Gravity. Around the immediate vicinity of *S. microphylla* trees large quantities of seed were found both on the surface of the soil and beneath the surface. This reinforces the observation that high numbers of seeds are released as individual entities from disintegrating pods on the tree. However, a large proportion of this seed would also come from seed pods disintegrating on the ground after falling from the trees. Most seed ends up being dispersed only short distances from the parents especially when growing in places surrounded by thick vegetation such as forest environments. In forests or dense grasslands, gravity dispersal would probably be the only mechanism employed.

(b) Wind. As shown in the previous section, wind appears to be a very important part of the seed fall process for *S. microphylla.* In pre Polynesian times, when forest covered the plains, wind probably had little significance in the seed dispersal of many Canterbury *S. microphylla.* It is difficult to imagine how wind could disperse seeds or whole pods to large distances successfully in forested or even heavily grassed environments. However, Godley (1975) noted the significance of wind in the dispersal of *S. microphylla* seeds even in the past. He states that the migration of kowhai seeds up river valleys and slopes is a relatively slow process, with the dry seeds or dry pods windblown for short distances across the ground. Also, for trees established on lower river terraces which are relatively new surfaces, wind may have acted more strongly on dispersal of seed pods. This is because these surfaces are more open and the vegetation much more sparse. The artificial removal of vegetation from the plains and surrounding hills, has probably meant that wind now has larger role in dispersing the pods throughout the Canterbury region.

(c) Water. Water appears to be the main mechanism used by *S. microphylla* for long distance dispersal, and dispersal of *S. microphylla* seed by water has been documented mainly by Godley and associates (eg. Sykes and Godley (1968), Markham and Godley (1972), Godley (1975)) but also Murray (1986).

There is substantial evidence to support the view that *S. microphylla* seeds have evolved for water dispersal. The most prominent evidence is the apparent buoyancy of some seeds which probably aids their transport over long distances by river and ocean currents. A sample tested for buoyancy showed that about 10% of *S. microphylla* seeds used in this study were buoyant in water. Tetrazolium tests have proven all buoyant seeds tested so far to be viable. Godley (1975), found that up to 27% of seeds were buoyant in his studies of the species and Sykes and Godley (1968) attribute this buoyancy to the lower density of the embryo.

Water is not only a dispersal mechanism for individual seeds. It was discovered that the dry seed pods floated very successfully on the surface of water. Seeds getting into waterways inside intact seed pods would probably have a distinct advantage in moderate to long distance dispersal. Not only would the surface floating pods travel longer distances in shorter time (due to the reduced resistance compared to below the surface), but the pods would protect seeds from the potentially destructive riverbed and obstacles in a river system. Unfortunately the length of time pods stay afloat is not known and has not been documented in any of the literature. Despite this the low moisture content and very buoyant nature of the seed pod suggests they remain afloat for reasonably long periods of time.

The long distance dispersal of *S. microphylla* seeds by water may also be a possible explanation for the development of the tough impenetrable testa. It is feasible that the tough testa is a long term dormancy mechanism, preventing the penetration of water, enabling seeds to be carried long distances without imbibing. However, the question of why all *S. microphylla* seeds are not inherently buoyant is yet to be answered. Experiments on the time *S. microphylla* seeds, both buoyant and non-buoyant, can spend in water, have shown that seeds soaked six months in pure water had still not imbibed (personal obs.). Godley (1975) showed that *S. microphylla* seeds could survive up to at least eight and a half years in salt water, with the majority of seeds remaining viable. Other evidence of compatibility with water dispersal is that *S. microphylla* frequents riverbanks, estuaries and lake margins, for example the Waitangiroto river depicted by Godley (1975), and the Waimakariri river (this study). The presence of plants in these

environments suggests seeds are deposited there by water. On the Canterbury plains most of the trees existing today, grow along the lower river terraces of the major river systems in relatively recently deposited alluvium. It is probable that these individuals arrived in these places as seeds deposited with the sediment. However, the presence of some individual trees remote from water courses poses the largely unsolved problem of how they got to such sites.

(b) *Sophora prostrata*

S. prostrata seems to grow almost exclusively on open country on bluffs and banks, sometimes at high altitudes. It does not seem to have any affiliation for long distance dispersal, and in general dispersed seeds remain close to the parent plant (personal obs). Water is generally sparse where they grow and the seeds have not evolved as suitable to water dispersal as those of *S. microphylla*. *S. prostrata* appears to rely consistently on the action of gravity or wind to disperse seeds, particularly in high altitude environments like the Port Hills. Buoyancy does not exist at all in *S. prostrata* seeds, and an impenetrable seed coat was seen in only 80% of individuals (cf ~100% in *S. microphylla*). This, in relation to germination without scarification, is discussed more fully in Chapter 8.

The pods of *S. prostrata* are light, and the seeds small, with few seeds per pod. They blow across open ground easily in strong winds, and pods disseminate much more readily. Logically there would be a reliance on quick release of seeds in open environments so that the pods containing the seeds were not blown too far away from sites suitable for germination. This, aided by a lack of dormancy (discussed for *S. prostrata* in Chapter 8) in some seeds to promote quick germination would mean seeds could establish quickly and more efficiently.

The tightly interlaced branches and prostrate nature of the plant however, often means that seed pods are trapped and disintegrate inside plant canopies before dispersal away from the plant can take place. Also, winds strong enough to carry the seeds from the parent plant do not always occur. Under these circumstances gravity generally takes over and seeds and seed pods travel

downslope, many of which are lost in the bush or streams. Some will probably germinate on suitable environments at lower altitudes and genetic research may prove that many individuals are genetically related over an altitude gradient. Some seeds are arrested by the thin humus layer and network of stems underneath mature "clump's' of plants. It is possible that these seeds may provide a source of regeneration during the senescence of older individuals in a stand.

4. Seed Banks

(a) *Sophora microphylla*

Two types of seed bank were recognised for *S. microphylla* in this study. The first was soil seed banks which are extensive around the immediate vicinity of the majority of individuals, and the second was the tree seed banks which has already been discussed in the section on seed fall.

The depth distribution of *S. microphylla* seeds in the soil seed banks was studied using soil profiles. To date there has been no documentation to substantiate whether *S. microphylla* seeds germinate from within the soil seed bank. The apparent close 'bunching' of individuals within *S. microphylla* populations and presence of seedlings under mature individuals after heavy spring rains suggests that this may be the case. To determine whether germination was taking place, there was close examination for imbibed and germinated seeds within the seed bank profiles.

Seed Bank Profiles

Methods

Two seed bank profiles were done under different trees. It was ensured that both trees were of approximately the same size and seed yield. To get the best idea of the depth distribution of seeds in the soil, profiles were done directly under the canopy in the lee of the northwest wind, where the seed fall was likely to be the most prolific. Each profile was divided into three separate

sections, 0-10cm, 10-20cm and 20-30cm. After removing the soil from each of these zones with a spade and placing it in a 40x40cm white plastic tray, seeds were sorted from the soil and counted. It was easy to separate the seeds from the soil in this way because of their bright yellow seed coats. A careful note was made of the number of imbibed and germinated seeds found in each section of the profile.

Results

The results of seed distribution with depth are given in Table 5

Table 5. Number of non-germinated, imbibed and germinated seeds in the soil seed bank of *S. microphylla*.

Profile No	0-10cm	10-20cm	20-30cm	No Imbibed	No Germ
1	252	18	2	0	4
2	150	3	1	0	1

It can be seen that the vast majority of seeds are contained within the top 10cm of the profile. The reason for this is not clear, because for the majority of individuals, substantial numbers of new seeds are being put into the soil seed banks each year. One explanation may be that seeds which become deeply imbedded in the soil encounter anaerobic conditions, fail to germinate because of this and subsequently decompose. These profiles were done in late September after a period of rain. This was fortunate as the 5 germinated seeds found in the profiles may not have been recorded if it were not for this recent moisture. The presence of germinating seeds in the soil profile is proof that with time *S. microphylla* seed coats will deteriorate in the soil enough to facilitate imbibition and germination. All of these germinating seeds were found in the top 10cm of soil. This is probably not a factor of depth but rather that the vast majority of the seeds were found in this zone. Experiments have been done on germination of seeds in varying depths of soil and these will be discussed later.

(b) *Sophora prostrata*

S. prostrata does not appear to maintain any substantial seed bank either on the plant or in the soil, although as already indicated from the section on seed dispersal some seeds do reside in the humus layer below groups of plants and these appear to be viable (from tetrazolium tests). However, these seeds do not seem to become buried to any appreciable depth, presumably due to the slow but consistent loss of seed and loose humus matter to gravity from the slopes on which it grows. There appears to be no immediate benefit in *S. prostrata* having a seed bank although assimilation of the seeds into the soil away from the parent plant must be important for its establishment.

5. Discussion

It seems that *S. microphylla* benefits from two of the most predominant features of the Canterbury landscape; wind and large river systems. Wind has a greater role to play now than it did in the past with the absence of the forest and extensive grasslands that once existed on the plains. The amount of seed lost or retained by *S. microphylla* seems to be a factor of wind, and probably varies from year to year depending on the wind patterns. It is possible that in years when winds are frequently strong, regardless of its direction, the vast majority or even all seeds may be lost from some trees. Whether it is the plant's intention that some seeds be retained is difficult to judge although if retention occurs it must be assumed that there is some selective reason for this. Seed pods are probably subject to much greater dispersal area and dissemination today due to these increased wind effects. Although this may be the case, wind is probably still used primarily as the mechanism for removal of seeds from the tree rather than dispersal. It is also possible that due to the actions of wind many more *S. microphylla* seeds are finding their way into river systems in intact pods.

Over the course of time, large rivers such as the Waimakariri and Rakaia have moved back and forth across the plains. The rivers alter course constantly making the riverbeds and lower terraces extremely dynamic environments. The growth of large numbers of *S. microphylla* on these river margins has probably meant that, at times, stands are destroyed by flood events and the soil and alluvium which they grow in is relocated further downstream forming new terraces. The build up of large soil seed banks around mature individuals would suggest that many seeds are relocated with this material and may subsequently germinate to build new populations. Other seeds, which are not relocated with alluvium along river margins may undergo long distance dispersal, ultimately into and across the oceans. It is suspected that the seeds most likely to undergo this are the buoyant seeds, carried much more easily and quickly by river and ocean currents. Some non-buoyant seeds may be dispersed long distances in this fashion but their movement must be severely impaired by physical barriers and burial in riverbed and ocean sediments. Many *S. microphylla* seeds can be seen in the strand line on Waimari beach (near the mouth of the Waimakariri river) prooving that large numbers of seeds are finding their way to the ocean.

One of the greatest mysteries at this time is how the soil seed banks of species like *S. microphylla* form. It is difficult to imagine how such large quantities of seeds actually become buried in the soil particularly to relatively large depths such as 10cm and more. Floods and similar events may be a means to do this, although it seems that many seeds become assimilated into the soil without the aid of such occurrences.

S. prostrata seems to have much simpler seed fall and dispersal mechanisms relying almost solely on the action of wind to remove the seeds from the plant and disperse them in the immediate environment. *S. prostrata*, in general, has no appreciable seed banks, due mainly to the influence of gravity and relatively rapid germination. One of the main problems encountered by *S. prostrata* is establishment, with seeds having to be assimilated into the soil long enough to imbibe and germinate, often on steep slopes. *S. prostrata* tends to grow in isolated 'clump's' of individuals. It is suspected, like *S. microphylla*, that these 'clump's' probably resulted from the spread of seeds from one or two original parent plants, even though this study has produced no genetic

evidence to support this. In considering the plant's establishment circumstances it is not unreasonable to suggest that this is the case. There are probably very few seeds which establish away from main groups creating a reliance on regeneration within groups of plants to maintain population numbers at viable levels.

CHAPTER 7

THE INFLUENCES OF TEMPERATURE AND LIGHT ON GERMINATION AND EARLY GROWTH

1. General introduction

This chapter investigates the effects of temperature and light on the germination of *S. microphylla* and *S. prostrata* seeds. There are 6 different experiments presented, namely: optimum, minimum and maximum temperature; the effects of fluctuating temperatures; photoperiodicity; shading effects (light intensity); seed burial effects and a multi-factorial experiment on the combined effects of temperature and light. In all 6 experiments controlled growth environments have been used to generate the required temperature and light regimes. Germination was taken as the time of radicle emergence.

The number of *S. microphylla* seeds used in these experiments is different from that of *S. prostrata.* This is due to the much lower seed production in *S. prostrata* outlined in Chapter 3. The number of *S. microphylla* seeds used for each replicate in all experiments is 100, while only 30 *S. prostrata* seeds have been used. Consequently, the standard errors of *S. prostrata* results are significantly higher than those of *S. microphylla.*

Methods for scarification, surface sterilisation of the seeds and moisture regimes were the same for almost all experiments. Because of this, the methods for these procedures have been presented in a separate section (Section 2) rather than repeating them for each experiment. The methods concerning the number and types of treatments have been described individually under each experimental heading.

2. Methods of seed scarification and sterilisation, moisture regimes and data recording times

Scarification

The seeds of both species were artificially scarified by holding each individual seed with tweezers and chipping the seed coat with the corner of a dissecting razor blade. Caution had to be taken to make sure the embryo was not damaged in the chipping process. The size of the chip seemed to have no bearing on the rate of imbibition, but seed coats were broken on both lateral faces, so that regardless of the way seeds lay, water penetration was still able to occur. When chipping the seed, the integrity of each seed coat was completely broken so that the interior contents of the seed, usually the cotyledons, could be clearly seen.

Seed Surface Sterilisation

The seeds of both species were very prone to microbial breakdown by saprophytic fungi, particularly after imbibition had occurred. Therefore, surface sterilisation was performed on seeds in all germination experiments not involving soil. There seemed little point in sterilising seeds to be placed in the soil, due to its unsterile nature. To surface sterilise the seeds, they were placed in a 250ml beaker. A 1% solution of household bleach was added to them and the mixture stirred for a period of 20 seconds. Then the seeds were removed from the bleach solution and rinsed twice in sterilised water to remove any residue.

Moisture Regimes

Moisture regimes were similar to those in the immature germination experiments, with 15ml of water initially added to each petri dish. During the experiment the bases of the petri dishes were kept constantly moist, but not to the point of submergence which would create an anaerobic environment. The moisture in each petri dish was checked every day and this level was maintained as uniform as possible. The daily checking of replicates was important, particularly for those running at high temperatures as they dried out easily.

Timing and Length of Monitoring

There were a lot of experiments to complete in the time available for this project and therefore a time restriction had to be placed on the length of time each experiment was run. Consequently, all experiments, except those concerning seed burial and early growth, were run with a total allowable time limit of one month (30 days). Fortunately for most experiments this was satisfactory time to display anticipated trends. Seed burial and early growth experiments, due to their slower nature (described under the experimental heading), were run for 60 days and 48 days respectively.

It was envisaged that the time between recordings of results should be relatively short to make them as accurate as possible. Therefore, this time was confined to 3 days for all experiments.

Seeds were removed from the petri dishes as they germinated; this was to make future counting quicker, and also to try to avoid the effects of any natural chemicals released by germinating seeds, which may enhance or inhibit the germination potential of non- germinated seeds.

3. Experimental Methods and Results

Experiment 1. Optimum, Minimum and Maximum Temperatures

(a) Introduction

The main aim of this experiment was to examine the rate and magnitude of germination, under different temperatures, and thereby define optimum temperature ranges for germination of both species. The concept of an optimum temperature range for the germination of seeds was first introduced by Sachs (1860) (Bewley and Black 1982) and has since been used by many workers as a basis for the study of germination in relation to temperature.

(b) Methods

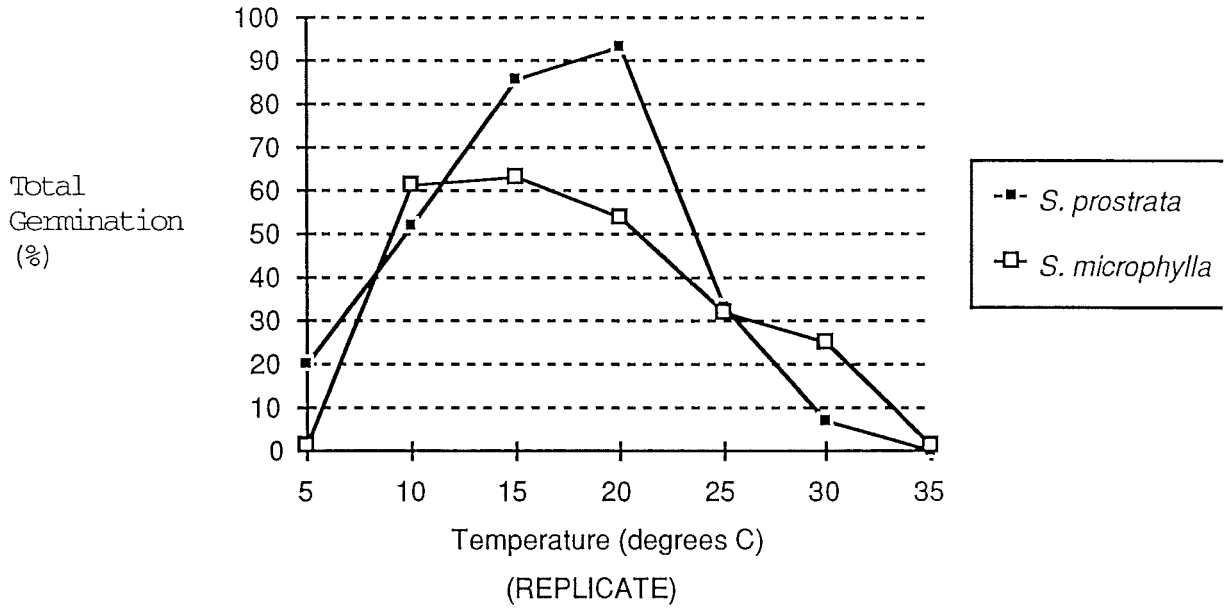
Once seeds had been scarified and surface sterilised they were placed on germination pads in 9cm petri dishes. The 100 *S. microphylla* seeds were divided into 4 lots of 25, each of which was put in a separate petri dish. The 30 *S. prostrata* seeds were all placed in one petri dish. All petri dishes were transferred to a Contherm CAT620 growth cabinet set at the required temperature which ran with 12 hours light and 12 hours darkness. Due to the absence of a thermogradient bar, each replicate had to be incubated separately. During the periods of lighting the replicates were exposed to the full light intensity available in the growth cabinets.

Results were pooled from this and other constant temperature experiments at the same light level enabling data to be collected for replicates run at constant temperatures of 5, 10, 15, 20, 25, 30 and 35^{o}C. The number of germinated seeds were recorded and then removed from the petri dishes. Moisture and recording procedures employed were as described above. The germination rate was calculated as the inverse of the time taken to complete germination.

(c) Results

The germination temperature curves for *S. microphylla* and *S. prostrata* are presented in Figure 7.

Figure 7. Germination percentages for *S. microphylla* and *S. prostrata* over a temperature gradient. **(Errors tabulated in Appendix 1 p129-130).**



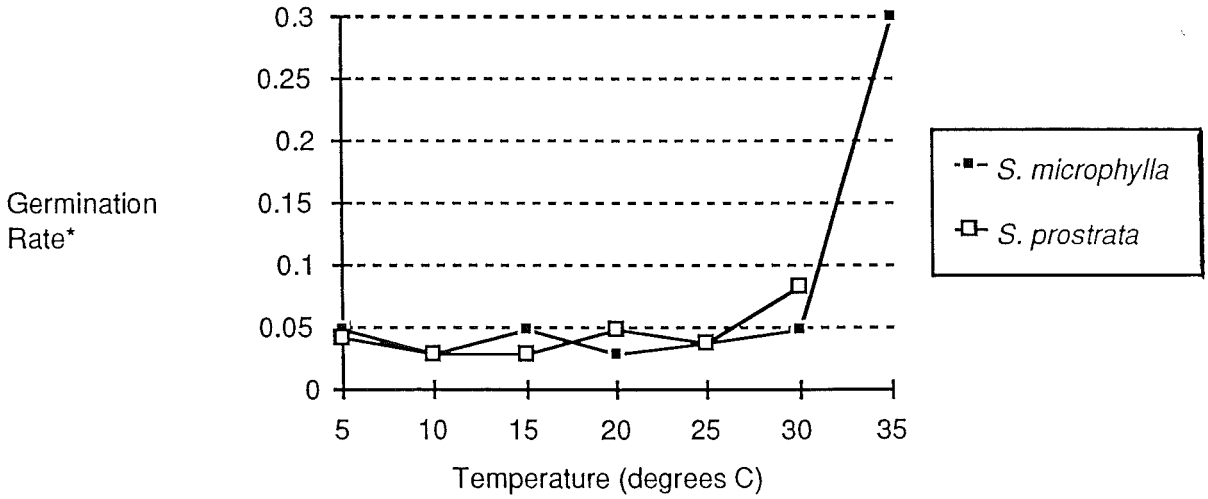
The germination responses indicate sensitivity to temperature for both *S. microphylla* and *S. prostrata*. Although the range at which seeds will germinate is relatively large for both species, spanning over 20°C for *S. prostrata*, their optimum germination ranges are quite narrow. The optimum temperature range for germination is between 10 and 20°C and this appears to be the same for both species. At this stage it is important to note that the time limit on experimentation may have been a significant limitation. I am sure that if the experiments had been allowed to run for a number of months, the optimum range of temperatures for germination would have been larger even though the rates of germination may have differed substantially. Nevertheless, there is an obvious trend illustrating temperature sensitivity.

Despite the apparent similarities of both species in the optimum germinating range, there appears to be some differences in the germination percentages at extremes. *S. prostrata* displays a greater ability to germinate at 5°C, while *S. microphylla* is able to germinate more readily in high temperatures. This might be expected, as *S. prostrata* is often confined to germinating at higher

altitudes at generally lower temperatures than *S. microphylla*. Ironically the peak in germination percentage is at a higher temperature for *S. prostrata* than *S. microphylla* (20°C cf 15°C). The reason for this is not clear, but it may be an error resulting from the use of fewer seeds in experimentation.

The germination rate is defined as the reciprocal of the time taken for germination to be completed. However, as already explained time limitations have probably affected these results. This is because, at lower temperatures particularly, it is thought that germination was not fully completed. The results have been included in the hope that they show general rate trends. Figure 8 shows these rates for *S. microphylla* and *S. prostrata.*

Figure 8. The germination rates of *S. microphylla* and *S. prostrata* over a temperature gradient.



* Germination rate = The inverse of the time taken to complete germination.

The germination rates for both *S. microphylla* and *S. prostrata* are very similar for all temperatures, once again drawing time limitation in the experiment into question. I think that if experimentation had been allowed to continue a much more characteristic low rate for low temperatures and high rate for optimum temperatures would have been achieved. This suggests that this graph is probably not a true reflection of rates. The rates at high temperatures are still

likely to be high because any germination that happens at temperatures above 20°C does so very quickly followed by rapid decomposition. Therefore, germination is completed and rates are high. The large jump in germination rate for *S. microphylla* at 35°C can probably be ignored as it is caused by the germination of a single seed within the first 3 days.

Interestingly, even in what was considered to be optimum temperatures for germination of both species, the number of days over which germination took place was large. This was seen in other experiments as well. Although many of the seeds for both species did germinate within the first 15 days in the optimum range, germination continued to occur, sometimes for the entire duration of the experiment. This makes the germination times large and the rate low. The presence of this time lag in germination suggests that the seeds of both *S. microphylla* and *S. prostrata* possess a dormancy mechanism which allows some imbibed seeds to germinate later than others regardless of how optimal conditions are. Further support of this hypothesis came from the results of one preliminary germination experiment on *S. microphylla* seeds in which some seeds germinated as soon as 3 days after imbibition, while others in the same replicate, if they survived microbial attack long enough, did not germinate until 4 weeks after imbibition.

Experiment 2. Temperature Fluctuation

(a) Introduction

This experiment was done to investigate the germination responses, in terms of magnitude and rate, of *S. microphylla* and *S. prostrata* seeds to sudden changes in temperature. Particular attention was given to the germination behaviour of seeds exposed to fluctuations in temperatures outside their optimum germination range. In nature, fluctuations in temperature are most likely to be due to diurnal temperatures although changes in the environmental conditions (for example from a northerly to a southerly wind) may cause similar effects.

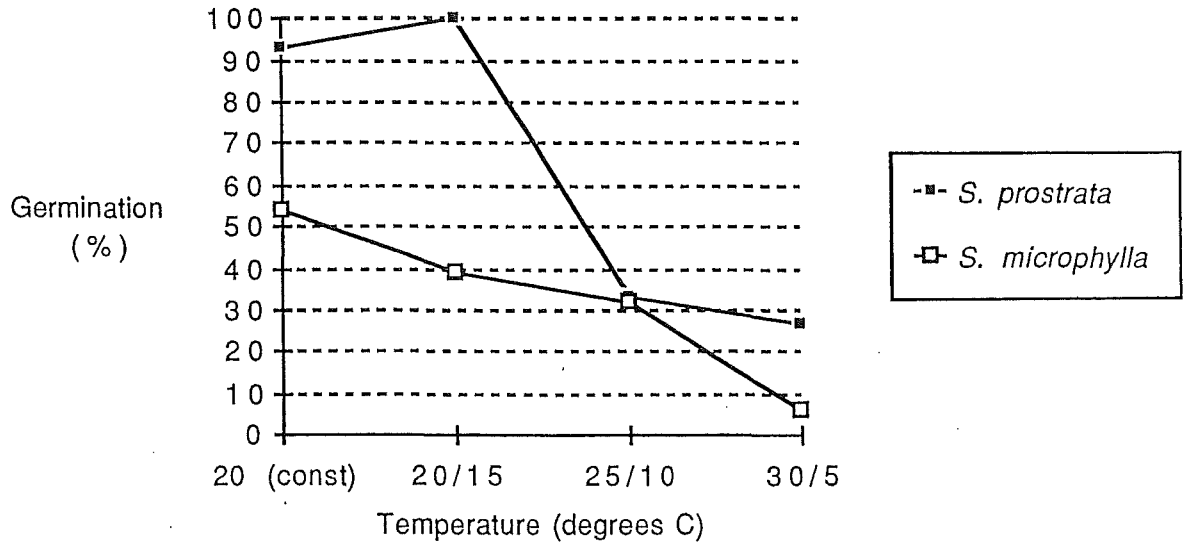
(b) Methods

After scarification and sterilisation, 100 *S. microphylla* and 30 *S. prostrata* seeds were placed in a Contherm CAT620 growth cabinet, programmed for 12 hours light and 12 hours darkness. This meant that there was no bias toward the effects of light. The lower temperature was run during the dark period and the higher in the light. The temperature fluctuations were between: (a) constant 20°C (no variation, ie. control), (b)20 and 15°C, (c) 25 and 10°C and (d) 30 and 5°C. The moisture regimes and time frames were the same as described above.

(c) Results

Both species responded to the fluctuations in temperature and the results for the percentage germination are shown in Figure 9.

Figure 9. Percentage germination of *S. microphylla* and *S. prostrata* under temperature fluctuation regimes. **(Errors tabulated in Appendix 1 p131-132).**



It can be seen that conditions favour germination significantly when temperature fluctuations are imposed on *S. prostrata* seeds. With temperature fluctuations within their optimum range (estimated as 10-20°C), the number of germinated seeds was very high (up to 100%). Compared to the germination percentages of seeds exposed to uniform temperatures, this is an improvement of up to 7%. This suggests that *S. prostrata* seeds may require temperature fluctuations to achieve optimum germination success. However, the steep and rapid decline in the germination percentage after temperature limits move outside the optimum range (25/10 and 30/5), reinforces the apparent sensitivity of seeds to temperature extremes during the germination process, even when exposure to these temperatures is only for a very short time.

Contrastingly, the results for *S. microphylla* show that germination percentages for seeds exposed to temperature fluctuation have declined compared to those exposed to uniform temperatures. Overall, the germination response of *S. microphylla* seeds to temperature fluctuation is poor. The fact that many *S. microphylla* seeds germinate after deep burial in the seed banks may be an explanation for this. Soil acts as a buffer against temperature fluctuation (Bradbeer 1988) and therefore *S. microphylla* seeds may be less well adapted to its effects. Alternatively, the greater probability of higher numbers of *S. prostrata* seeds remaining unburied means they should be more adapted to its effects.

Experiment 3. Photoperiodicity

(a) Introduction

A number of workers have shown that the germination behaviour of seeds can be modified by the use of intermittent or daily illumination periods (eg. Bewley and Black 1982, Bradbeer 1988, Larcher 1980). The thick seed coat which *S. microphylla* and *S. prostrata* possess, and the germination of many seeds from within the soil, would logically indicate that light would not have a major role in determining their germination behaviour. However, this may change when the seed coat becomes scarified or broken and exposed to environmental elements.

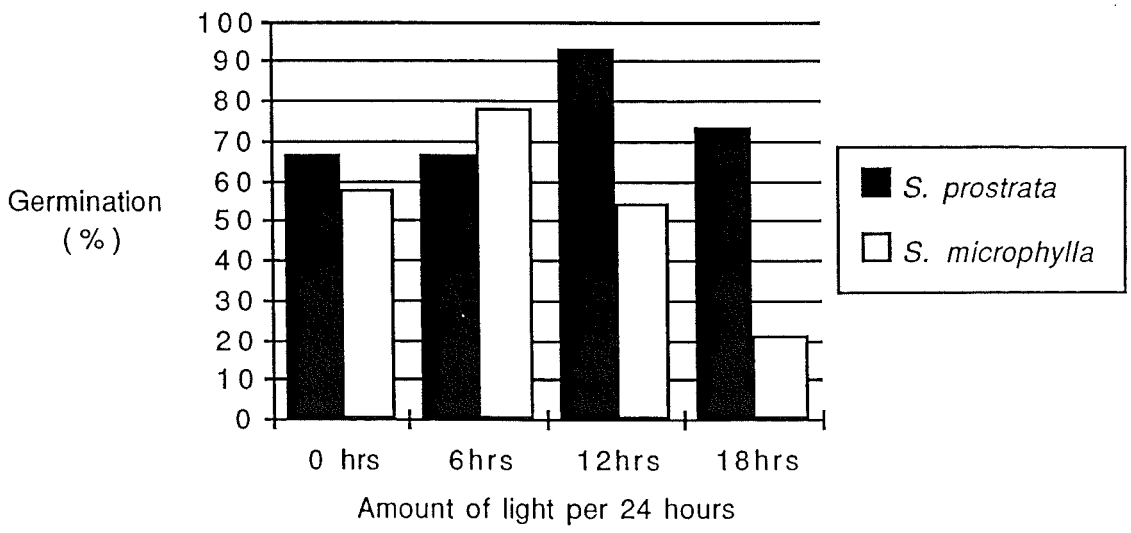
(b) Methods

Once again 100 *S. microphylla* and 30 *S. prostrata* seeds were scarified and sterilised in the manner described above. After this they were transferred to a Contherm CAT620 growth cabinet and maintained at a uniform temperature of 20°C. Light intensity was maintained at maximum within the growth cabinet to simulate pure sunlight conditions. The length of light each replicate received was then varied to simulate differing day lengths. The day lengths used were, 6 hours, 12 hours, 18 hours and complete darkness which was used as the control. Moisture regimes and time frames were as described above.

(c) Results

The results are summarised in Figure 10.

Figure 10. The germination response of *S. microphylla* and *S. prostrata* to photoperiodicity. **(Errors tabulated in Appendix 1 p133-134).**



The germination percentages of both species do appear to follow smooth curves, with maximums at the 6 and 12 hour periods for *S. microphylla* and *S. prostrata* respectively. This is significant

as the majority of daylengths during the year are between 6 and 12 hours long. The results suggest that there is a positive germination response associated with day length. However, I find the presence of such a response difficult to explain, especially for the majority of *S. microphylla* seeds, which spend their life in, and germinate from, the soil seed bank in lightless conditions. This is emphasised by the good germination success in the dark, which accords with observations that a high number of seeds germinate after burial.

There seems to be a marked negative response of *S. microphylla* to the effects of increasing daylight, particularly at long daylengths. Bewley and Black (1982) noted that light can inhibit the germination of some seeds. If *S. microphylla* prefers to germinate after burial in the soil, then it is possible that its germination behaviour may be inhibited by prolonged periods of exposure to light, particularly if scarification has taken place. The *S. microphylla* seeds germinating under the 18 hour day regime revealed one of the lowest germination percentages seen in germination experiments to date. This supports the inhibition theory for this species, although it is not conclusive evidence. More long-term experimentation is required to establish this.

In general it is probably safe to state that under normal conditions (about 10 hours daylight per 24 hours) daylengths would not play a large role in the germination response of the seeds of either species.

Experiment 4. Shading Effects

(a) Introduction

This experiment addresses two aspects of the ecology of *S. microphylla* and *S. prostrata*. It looks firstly at the germination of their seeds under different shading regimes, and secondly at the effects of different intensities of light on the early stages of plant establishment, growth and foliar development. The main aim of these experiments was to establish how well the seeds and young

seedlings of these two *Sophora* species perform in poor light environments (such as those found in forested or heavily grassed areas) when germination and establishment is taking place.

(b) Methods

(i) Shading regimes and Germination

In investigating the effects of different shading regimes on germination, seeds were scarified and surface sterilised in the normal way. For each of the 5 replicates 100 *S. microphylla* and 30 *S. prostrata* seeds were used with, the 100 *S. microphylla* seeds being divided into 4 lots of 25 as described in previous experiments. Instead of being transferred to a growth cabinet, replicates were transferred to a glasshouse where the temperature was maintained as near as possible to a uniform 20°C and ordinary sunlight was used as the light source. To vary the intensity of the sunlight reaching the seeds, differing thicknesses of shade cloth were used. Progressive decreases in light intensity were obtained by covering replicates with either 1, 2 or 3 layers of shade cloth. The replicates in these three shaded environments were labelled shade 1, shade 2 and shade 3 and the light intensity, measured with a quantum sensor, corresponded to 0.35, 0.20 and 0.09 microeinsteins m^{-2} sec^{-1}** respectively. These were the intensities of incident light reaching the seeds and seedlings under each regime. The unimpeded light intensity inside the glasshouse was 0.80 microeinsteins m^{-2} sec^{-1}. All readings were taken on clear sunny days. The fourth and fifth replicates were exposed to pure sunlight and pure darkness. Pure darkness was generated by placing the petri dishes in black polythene bags. This replicate was used as the control.

** Microeinsteins m^{-2} sec^{-1} equivelents are:

x 0.1 Watts m^{-2}

x 10 Lux

(ii) Shading effects on early growth and development

Height Measurements

In order to study the effect of different light environments on early growth and development after germination, seedlings had to be established. To do this, 10 scarified seeds of each species were sown, evenly spaced, at a depth of 10mm, in polystyrene boxes with dimensions 40cm x 25cm x 11cm (lxbxh). The boxes were filled with soil to the top allowing for unimpaired root development during the course of the experiment. The soil used was a commercial organic mix of topsoil and organic matter called Bio-Blend. Used by commercial seed propagators, it was chosen to provide good soil structure and fertility for the growing seedlings. The same shading regimes used for the shaded germination experiments were used in this experiment, with a simple wooden frame constructed over the boxes to support the various layers of shade cloth. The shade cloth was secured to the frames using drawing pins. The replicate run in the dark was placed inside 2 black plastic rubbish bags, one inside the other. A similar frame was constructed on the inside of the bag to keep the plastic from congesting the seedlings. This was important in this experiment as most seedlings became very etiolated. Enough moisture was applied to the replicates to keep the soil moist to the touch, but not saturated.

Only 10 seedlings of each species were established under each shading regime to maintain practicality in measurement. Although this is not a statistically significant number the experiments were only designed to give an idea of seedling response to light. Consequently errors are very high and their significance questionable. For this reason they have not been included.

Every 3 days the height of each germinated seedling was recorded. The height of each seedling was taken as the total length of the main leader between the soil and the apex of that leader. Measurements were made by gently placing the main leader against a ruler and noting the measurement to the nearest millimetre. Unfortunately, the germination times of most seeds varied quite substantially, so a note had to be made of the time of germination of each seedling. Using

this as a starting point, measurements could be made for exactly the same length of time for each seedling. For each time period (3, 6, 9 days etc), the heights of the 10 seedlings in each replicate were averaged to provide an average height growth figure. These figures were used as the basis for the graphs presented in the results. The time limit of 30 days was not enough to germinate the seeds and make an accurate assessment of early growth. Consequently the heights of each seedling were taken at 3 day intervals, for a period of 48 days, from the time that the seedlings could be practically measured. Practical measurements could be made when the seedlings had reached a height of 7mm.

Leaflet Counts

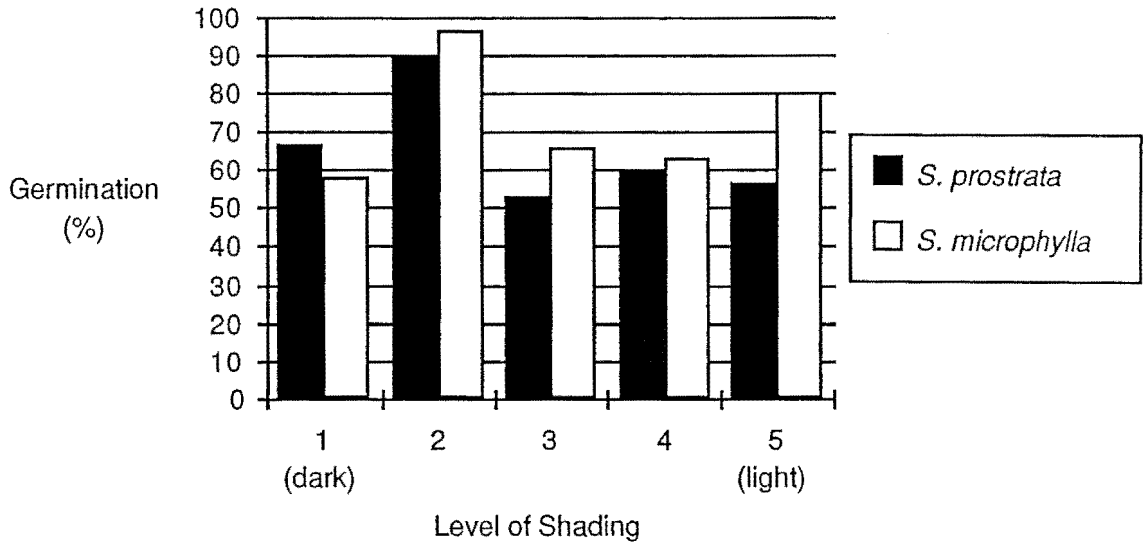
The leaflets on each leaf were convenient 'units of foliage' which could be counted on a regular basis to monitor the amount of foliar development. A more conventional method of assessing this was to measure leaf areas at certain ages. However due to the largely divaricating nature of seedlings, especially *S. prostrata*, the use of leaf area equipment was difficult. This, coupled with time limitations meant measurement of leaf areas was not a practical path to follow. However, it was considered important to get an idea of foliage behaviour under different light intensities. Consequently, the number of leaflets on each plant were counted every three days and this number averaged in a similar fashion to that of the height measurements.

(c) Results

(i) Shading effects on germination

The shading of seeds seemed to have little effect on their germination for 4 out of the 5 replicates. The germination percentages attained for both species under each shading regime is given in Figure 11.

Figure 11. Germination of *S. microphylla* and *S. prostrata* seeds under different shading regimes. 1=darkness; 2=shade 3 (0.09 microeinsteins m^{-2} sec^{-1}); 3=shade 2 (0.20 microeinsteins m^{-2} sec^{-1}); 4=shade 1 (0.35 microeinsteins m^{-2} sec^{-1}); 5=daylight (0.80microeinsteins m^{-2} sec^{-1}). **(Errors tabulated in Appendix 1 p135).**



All of the germination percentages except those for the most heavily shaded regime (2) are approximately the same. It is not understood why there is a significant difference in the germination percentages for shading regime 2. This result is unusual, because it also displays an extremely high germination percentage for *S. microphylla* (96%), a figure seen nowhere else in experimentation even when conditions were considered optimum for germination. It is possible that this heavily shaded environment is equivalent to light conditions just below the surface of the soil, or alternatively similar to that in heavily forested or grassed areas. Many *S. microphylla* seeds are probably adapted to germination in heavily shaded environments which may therefore be optimum for germination. The similar result attained for *S. prostrata* suggests that a similar effect may be taking place with its seeds as well.

It seems that the results are indicating either a very specific germination response to light intensity or an anomaly. The pronounced response of young seedlings of both species to different light

intensities (discussed in the following section) would tend to support the former, although the behaviour of seeds and young plants is often mutually exclusive. The relatively high germination percentages in the other replicates suggests that there is not conclusive evidence that there is a specific germination response to light intensity. Good quantities of seed, in both species are able to germinate whatever the light conditions are. This is a significant result, as it means the seeds of both species are versatile in the type of environment they are able to germinate and establish in. This shows that the seeds of *S. prostrata* could actually germinate in forest environments but the apparent lack of plants in these areas indicates subsequent failure due to competition.

(ii) Early growth and development in young seedlings

Height growth

Seedlings of both species responded to variation in light intensity. The results of height growth over time is given in Figure 12 for *S. microphylla* and in Figure 13 for *S. prostrata.*

Figure 12. Average height growth of 10 *S. microphylla* seedlings over time under differing shading regimes; daylight (0.80microeinsteins m^{-2} sec^{-1}); shade 1 (0.35 microeinsteins m^{-2} sec^{-1}); shade 2 (0.20 microeinsteins m^{-2} sec^{-1}); shade 3 (0.09 microeinsteins m^{-2} sec^{-1}) and darkness (0.0 microeinsteins m^{-2} sec^{-1}).

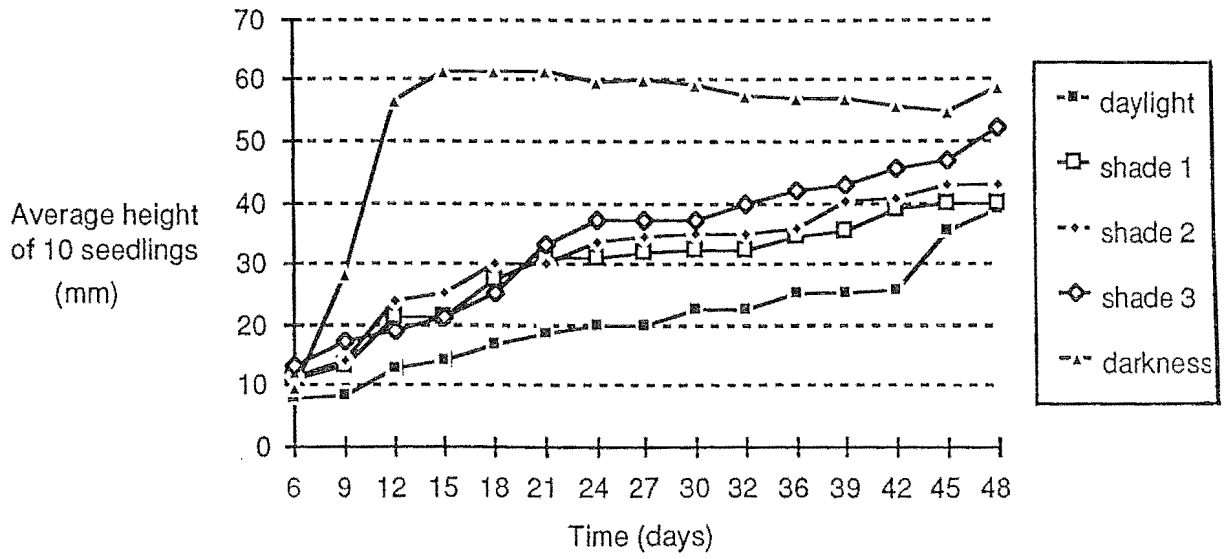
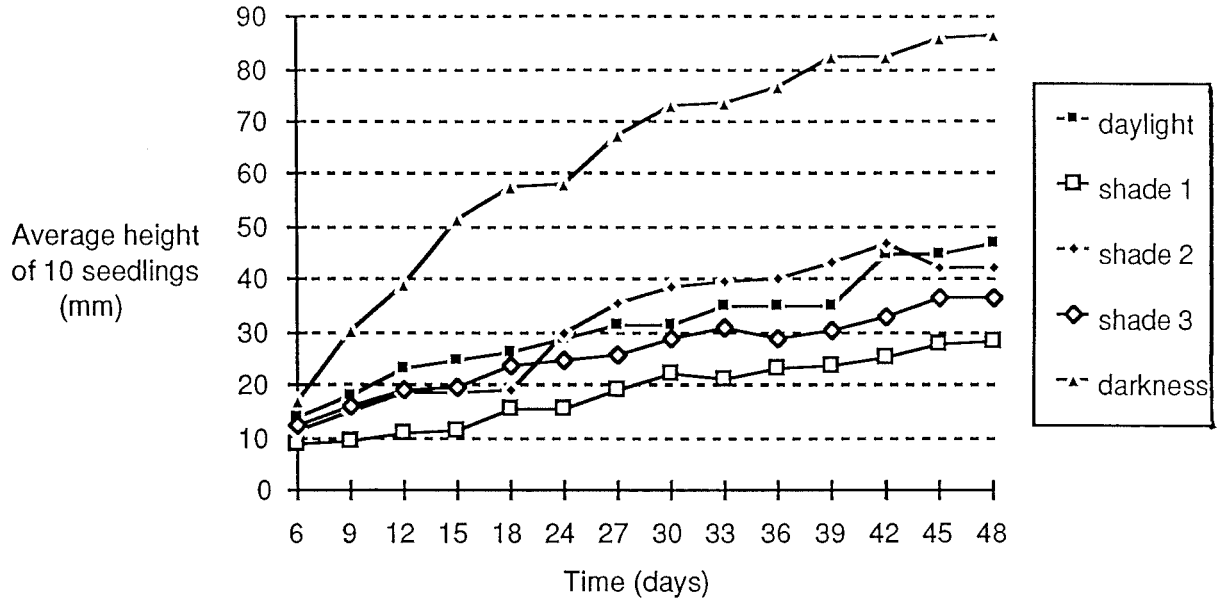


Figure 13. Averaged height growth of 10 *S. prostrata* seedlings over time under different shading regimes. daylight (0.80microeinsteins m^{-2} sec^{-1}); shade 1 (0.35 microeinsteins m^{-2} sec^{-1}); shade 2 (0.20 microeinsteins m^{-2} sec^{-1}); shade 3 (0.09 microeinsteins m^{-2} sec^{-1}) and darkness (0.0 microeinsteins m^{-2} sec^{-1}).



S. microphylla seedlings have a very characteristic response to changes in light intensity, with seedlings gaining height more rapidly as the growing environment becomes darker. Contrastingly, *S. prostrata* shows no obvious inclination towards this trend, except for a very pronounced response in the total absence of light. This suggests that *S. microphylla* seedlings are probably more competitive in poor light conditions than those of *S. prostrata.* The pronounced response in lightless conditions of *S. prostrata* seedlings is likely to be a characteristic associated with its initial escape from the soil and grass layers in which it establishes. It is interesting to note, however, that the *S. prostrata* seedlings exposed to pure sunlight, were second in height growth only to those run in the dark, indicating that good light conditions promote growth in this species. It must be noted that regardless of the light conditions all seedlings including those of *S. prostrata* seemed to suffer no immediate ill effects from the lack of light. Even in the dark, seedlings remained alive throughout the course of the experiment (approximately 7 weeks), although some dieback at the tips of the plants began to occur toward the end. As might be expected, in the dark there was an almost total absence of chlorophyll and few leaves were

produced on either species. This coupled with the long straggling stem shows distinct etiolation and that the majority of resources are going into the act of finding the light through elongation of the stems before photosynthetic tissues are produced.

One of the major factors enabling the seedlings of both species to stay alive for so long in poor light conditions could be that the seeds are large, containing substantial food reserves. An experiment was done very early in the experimental work to assess how long *S. microphylla* seedlings could live solely on the reserves of their seeds. Of the 10 seeds germinated in a petri dish, 8 lived for more than 2 months and 3 lived for more than 6.5 months. Although a similar experiment was not conducted for *S. prostrata* there is little doubt that results would be similar, judging from the size of seeds. Therefore, seedlings may be able to live for very long periods in the dark purely on the resources of their seeds; a consequence of not having to manufacture their own food through photosynthesis.

Another interesting point to note is that after germination has taken place and the remaining portion of the seed has discarded its seed coat, so long as the seed is exposed to light, the surface of the cotyledons turn green and become photosynthetic. This was seen in the seeds of both *S. microphylla* and *S. prostrata*. The germination of both *S. microphylla* and *S. prostrata* seeds appears to be chiefly epigeal (although the cotyledons of some deeply buried seeds may not emerge on germination) and photosynthetic cotyledons are characteristic of seeds which have epigeal germination (Bradbeer 1988). For seeds germinating on or near the surface of the soil, the extra surface area for the production of food resources would be an advantage to the plant, particularly during establishment. This phenomena was seen on seeds in all light conditions except those in the dark.

Foliar Development

After counting the number of leaflets and averaging this quantity over time I was able to assess trends in foliar development for the various shading regimes. These trends are displayed in figure 14 and 15 for *S. microphylla* and *S. prostrata* respectively.

Figure 14. Development of leaflets over time for *S. microphylla* under different shading regimes; daylight (0.80microeinsteins m^{-2} sec^{-1}); shade 1 (0.35 microeinsteins m^{-2} sec^{-1}); shade 2 (0.20 microeinsteins m^{-2} sec^{-1}); shade 3 (0.09 microeinsteins m^{-2} sec^{-1}) and darkness (0.0 microeinsteins m^{-2} sec^{-1}).

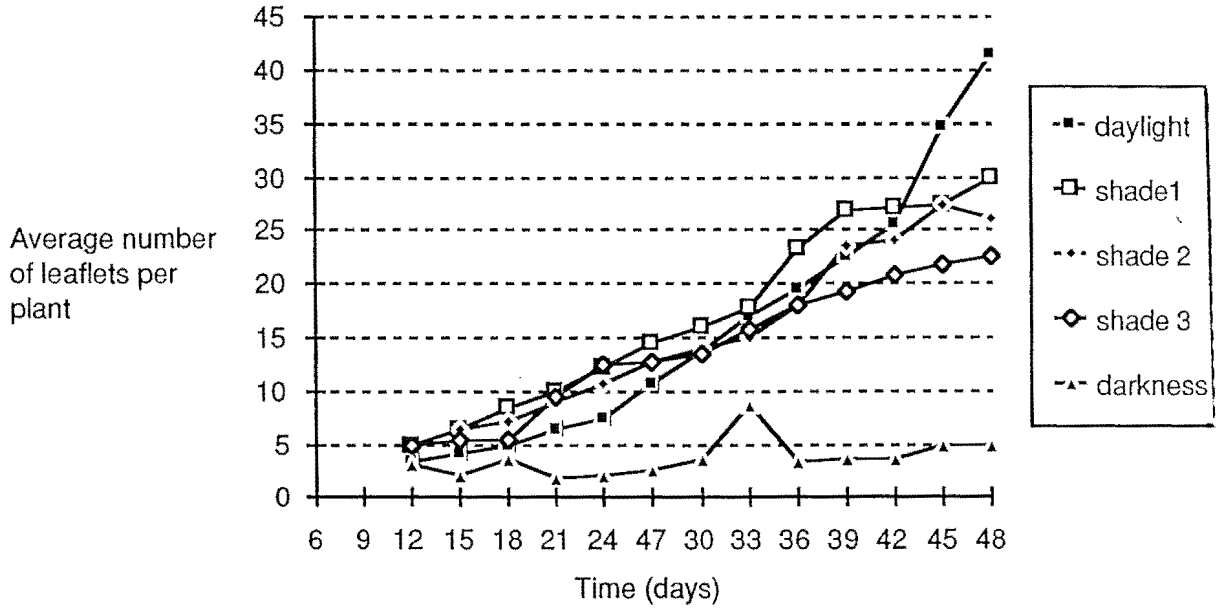
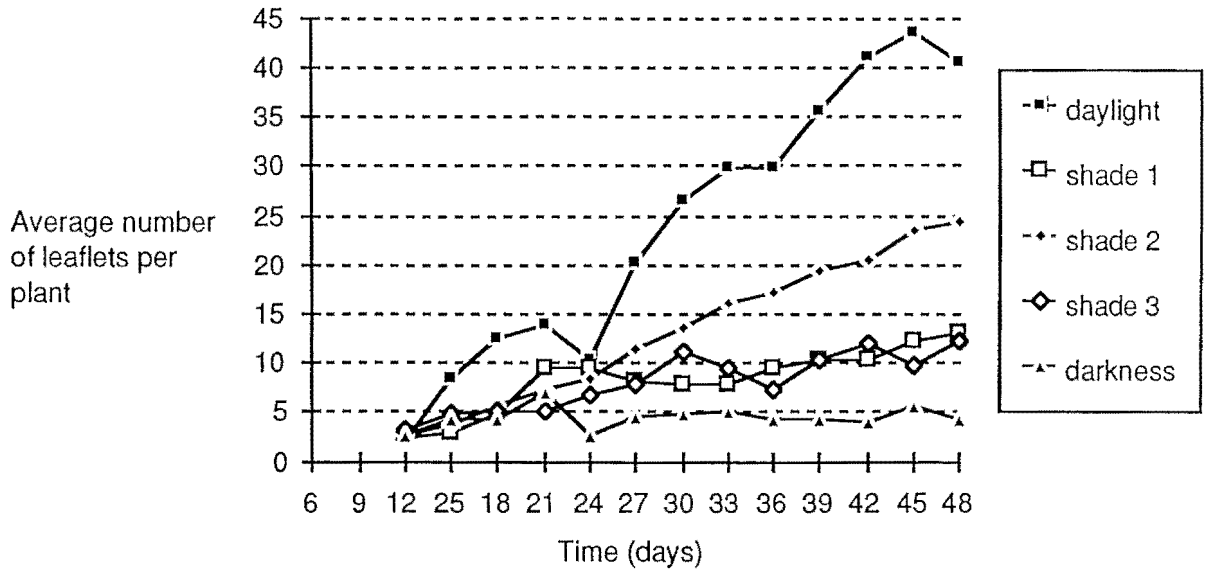


Figure 15. Development of leaflets over time for *S. prostrata* under different shading regimes; daylight (0.80microeinsteins m^{-2} sec^{-1}); shade 1 (0.35 microeinsteins m^{-2} sec^{-1}); shade 2 (0.20 microeinsteins m^{-2} sec^{-1}); shade 3 (0.09 microeinsteins m^{-2} sec^{-1}) and darkness (0.0 microeinsteins m^{-2} sec^{-1}).



At the beginning of this experiment it was expected that the more shaded seedlings were the more foliage they would produce to compensate for the lack of light. However, the graphs show a distinctly contrasting trend. It seems that for both species the development of foliage in the early stages of growth is dependent on the amount of light being received by the seedlings rather than the lack of it. The general trend both species show by the end of the experiment (after 48 days) is that seedlings in pure sunlight display the greatest foliar development, although greater time is needed to show this in *S. prostrata*. Foliar development becomes progressively less with reduction in light intensity until it is virtually nil in the darkness suggesting that foliar initiation and development is dependent on light intensity. This is further evidence that these plants undergo distinct etiolation. It is likely that both species would have a distinct advantage on establishment because they are able to etiolate. This adaptation would enable them to maintain a state of foliar dormancy until they are exposed to light and can capitalise on its energy resources.

Experiment 5. Seed Burial

(a) Introduction

Soil seed banks are a very prominent part of the ecology of *S. microphylla* and most seeds germinating in close proximity to the parent trees do so from within the seed bank. Although *S. prostrata* does not have the extensive seed banks characteristic of *S. microphylla*, germination probably occurs after assimilation of seeds into the soil. *S. microphylla* and *S. prostrata* seedlings germinating in the dark can grow to substantial lengths (13 cm in some cases). This suggests an ability to germinate from deep within the soil profile. However, there are a number of different factors which limit the germination of deeply buried seeds. For example, with many species the amount of oxygen and light reaching seeds in soil seed banks rapidly diminishes with depth, thereby compromising their germination ability (Mayer and Poljakoff-Mayber 1982). This is due to the physical barrier to oxygen and light penetration given by the soil, as well as the effects of soil microbe populations on oxygen concentrations. High carbon dioxide (produced by the decomposition process) is known to be inhibitory to the germination of some seeds (Bewley and Black 1982).

This experiment looks at the germination response of *S. microphylla* and *S. prostrata* seeds at different depths in the soil to investigate optimum germination and establishment characteristics from within the soil profile.

(b) Methods

After scarification, 100 *S. microphylla* and 30 *S. prostrata* seeds were sown at 6 different depths in Bio-blend mix placed in polystyrene boxes. These depths were: surface (control), 5mm, 10mm, 20mm, 50mm and 100mm. The soil was kept moist, but not wet, and the boxes were placed in a glasshouse and maintained at 20°C. Every 3 days the number of emerged seedlings

in each replicate was recorded. To allow enough time for emergence of the more deeply buried seeds, this experiment was run for 60 days instead of 30 and to avoid too much disturbance of the soil, seedlings were not removed as they emerged.

(c) Results

Figure 16 and 17 presented below show the emergence and establishment of *S. microphylla* and *S. prostrata* seedlings respectively and display two pieces of information. Firstly, they give an indication of the magnitude of germination at various burial depths and they show the number of successfully established seedlings over time.

Figure 16. The emergence and establishment of *S. microphylla* seedlings over time. **(Errors tabulated in Appendix 1 p137)**

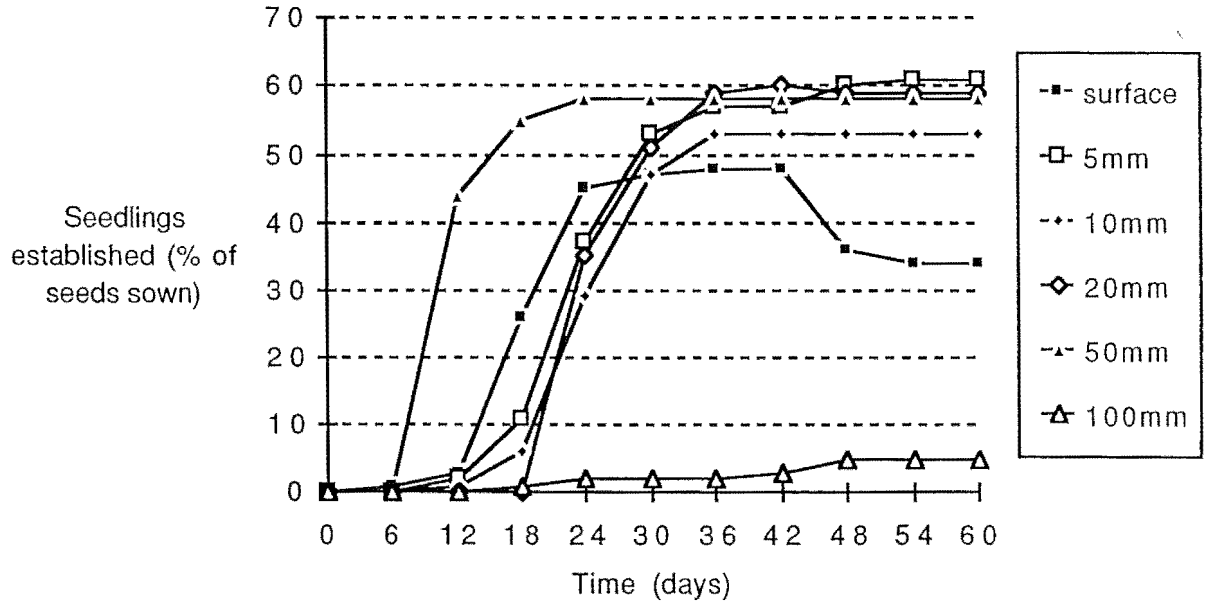
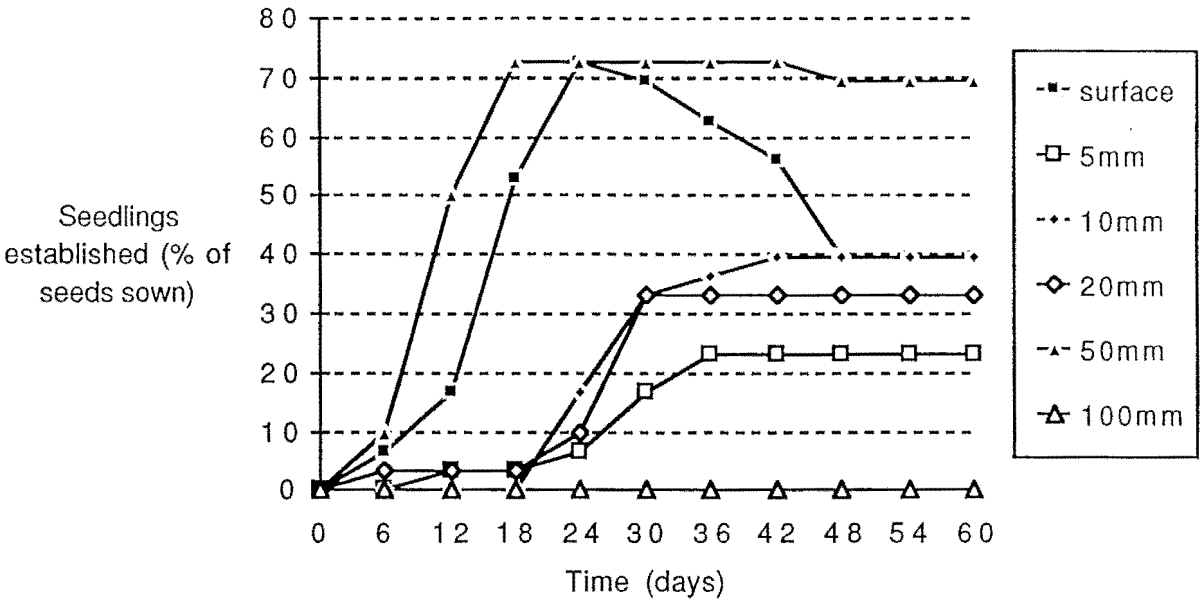


Figure 17. The emergence and establishment of *S. prostrata* seedlings over time. **(Errors tabulated in Appendix 1 p136)**



From the graphs it would initially appear that *S. prostrata* is more sensitive to burial than *S. microphylla*, with noticeably larger variation in establishment under different burial regimes. However, there does not seem to be any consistent trend or pattern to the *S. prostrata* results. For example, the establishment of *S. prostrata* seedlings from seed buried at 10mm seems to be more favourable than those at either 5mm or 20mm. If trends were consistent the result would presumably be between the two.

Despite this, there are some interesting points to note. Firstly, for both species there is a greater rate and magnitude of establishment, at 50mm, particularly in the early phases of establishment. The reason for this is not understood, but it suggests that the seeds of both species will germinate and establish successfully from quite deep in the soil profile. This germination and emergence from deep burial seems to be particularly characteristic of *S. microphylla* and was reinforced by the emergence of 5% of sown seeds at a burial of 100mm. When looking at depth distribution of *S. microphylla* seeds in its soil seed banks, all seeds which had germinated had done so from between 50mm and 100mm down the profile. The absence of appreciable seed banks and litter

layers in the zones in which *S. prostrata* germinates seems to make the germination success of these seeds at 50mm unusual. There is no reason to believe however, that after assimilation into the soil the seeds do not remain dormant for long periods of time and eventually germinate from deep in the profile.

There is no doubt that the seeds of both *S. microphylla* and *S. prostrata* are suited to establishment after burial by soil or litter. One of the most striking trends in Figures 13 and 14 is the distinctive drop in the amount of established seedlings after about 18 days, for seeds sown on the surface of the soil. Understandably, there seems to be little impairment to the germination of these seeds, but their establishment is very poor. This indicates that if seeds of either species were to germinate on the surface of the soil, their chances of establishment would be lowered substantially. It may explain why the exposed, germinated seeds of both species turn photosynthetic. This may be an attempt to keep the seedling alive as long as possible while establishment takes place.

Experiment 6. Temperature/Light Interactions

(a) Introduction

Germination is often affected by the interaction of two or more environmental factors and the germination response of seeds to each of the factors individually may differ significantly compared to that when the factors work together.

This experiment looks at the germination response of both species to the interaction of temperature and light and makes a comparison to the germination response to temperature and light as individual entities.

(b) Methods

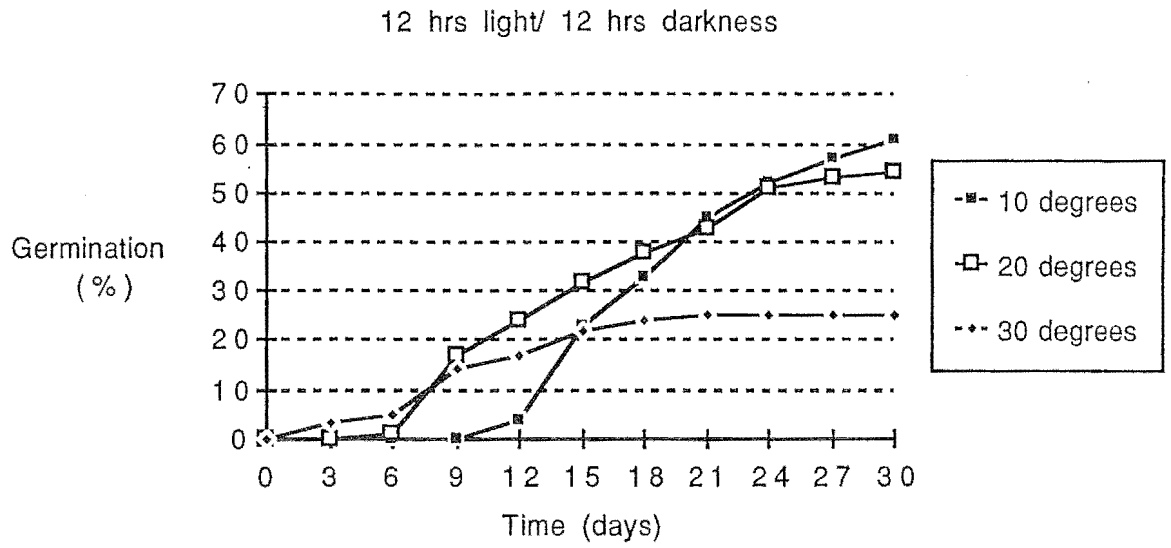
After scarification and surface sterilisation, 6 replicates of 100 *S. microphylla* seeds and 30 *S. prostrata* seeds were sown on 9cm germination pads in 9cm petri dishes. Three of these replicates were then subjected to 10°C, 20°C and 30°C respectively, and placed under a lighting regime of 12 hrs light and 12 hrs darkness. The other three were subjected to the same temperatures, but run in the dark with no light at all. These replicates were the controls. The various combinations of temperatures and light were achieved through the use of the Contherm CAT620 growth cabinets. Usual moisture and data collection procedures applied.

(c) Results

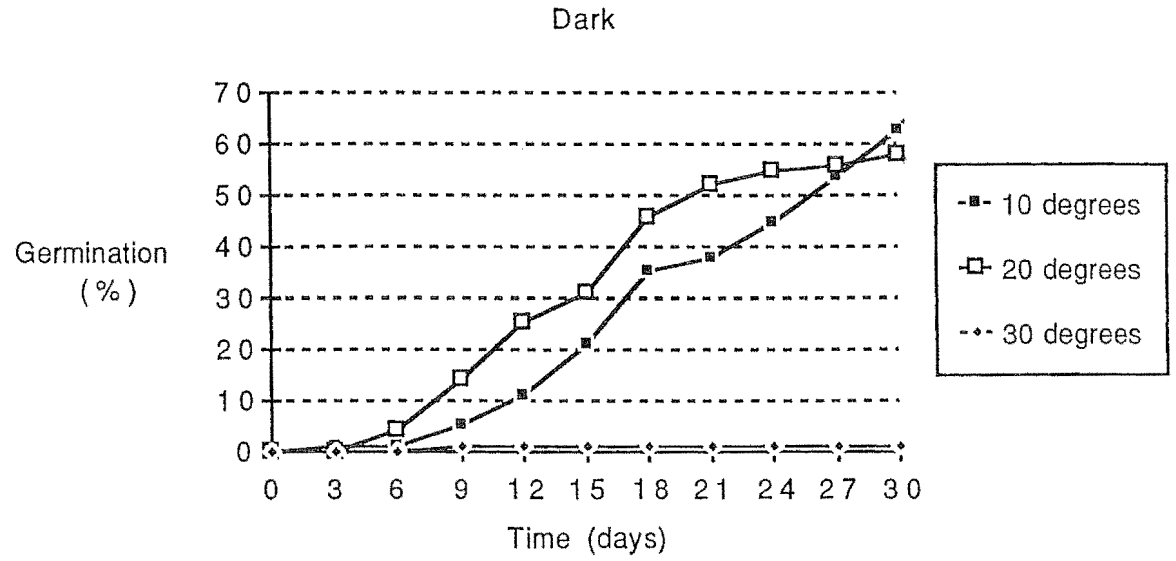
In Figure 18A and B comparisons have been made between seeds of both species germinated at different temperatures in 12 hrs light/12 hrs (A) darkness and in the dark alone (control) (B).

Figure 18A and 18B. The effects of the interaction of light and temperature on the germination of *S. microphylla* seeds. In (A) replicates were run in 12 hours light and 12 hours darkness. In (B) replicates were run in pure darkness and this was used as the control. **(Errors tabulated in Appendix 1 p140-141)**

(A)



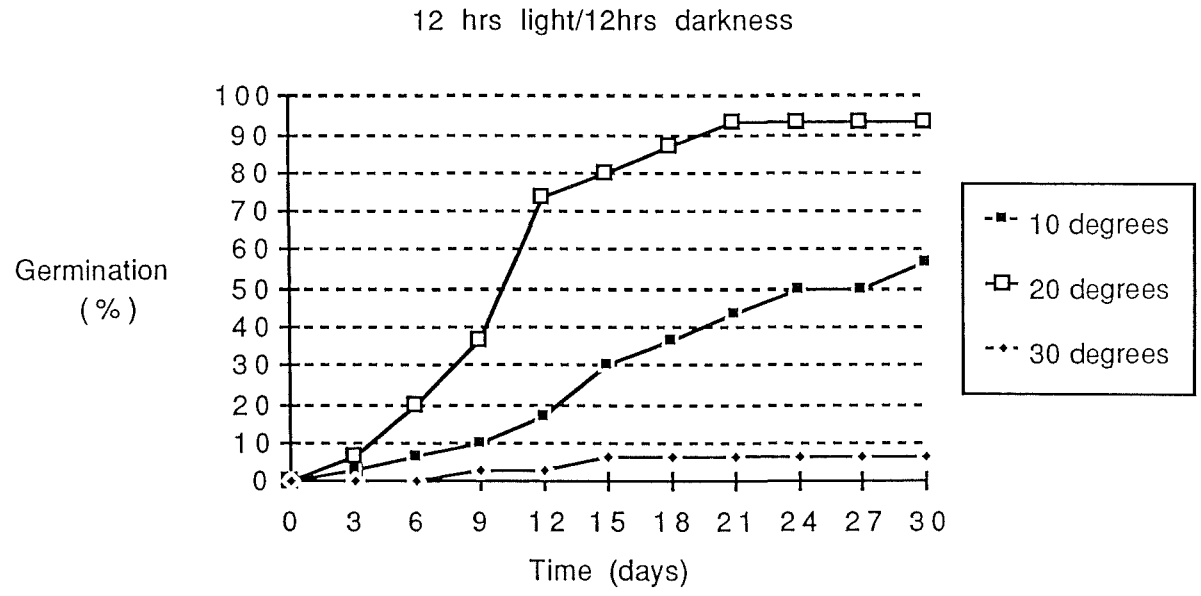
(B)



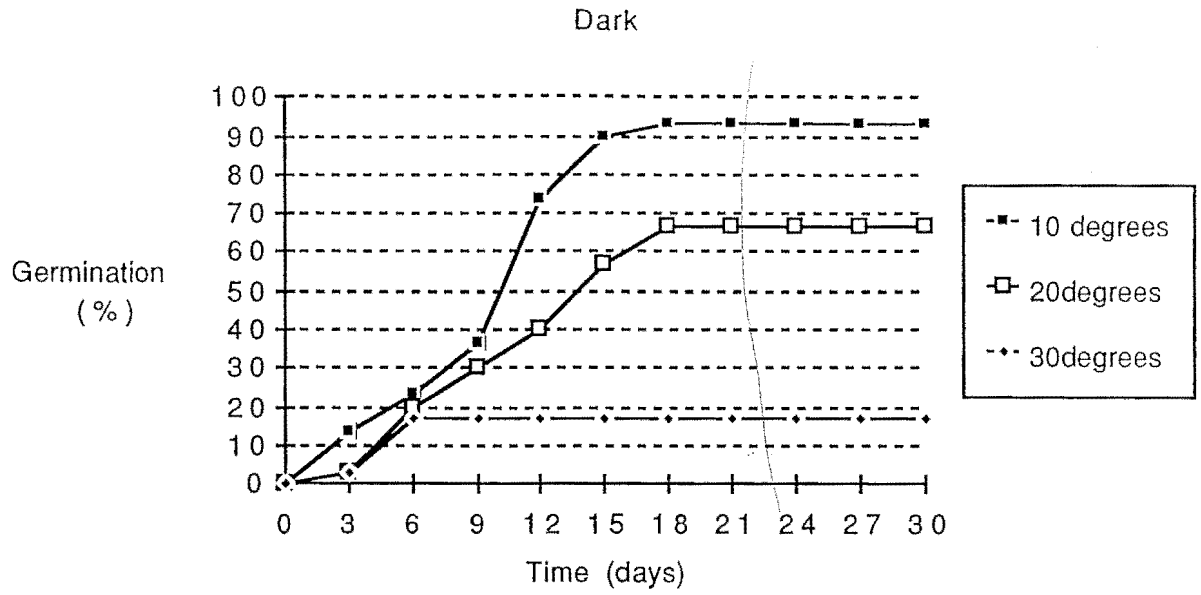
The main point to note is that light seems to increase the amount of germination of *S. microphylla* at higher temperatures, with 25% germination at 30°C in the light as compared with only 1% in the dark. The other trends stay approximately the same. However, these results are obtained with unrestricted moisture. In nature at times when temperatures are high and light is good, conditions are more likely to be dry, restricting germination. Furthermore, seeds of *S. microphylla* prefer to germinate after burial in the soil, which is lightless. It would only be if the soil was disturbed and seeds brought to the surface that light and temperature would be able to act together on the germination responses of the seeds. Figures 19A and B displays similar graphs for *S. prostrata.*

Figures 19A and 19B. The effects of the interaction of light and temperature on the germination of *S. prostrata* seeds. In (A) replicates were run in 12 hours light and 12 hours darkness. In (B) replicates were run in pure darkness and this was used as the control. **(Errors tabulated in Appendix 1 p138-139)**

(A)



(B)



It can be seen that the trends for germination of *S. prostrata* seeds at 10°C and 20°C in the lighted replicate almost totally reverse when light is taken from the system. This would suggest that in the dark, which is akin to being in the soil, germination percentages increase at lower temperatures for *S. prostrata*. This reinforces the apparent ability that some *S. prostrata* seeds have to germinate at low temperatures. There is an unexpected drop (26.7%) in germination percentage of seeds in the dark exposed to a supposedly optimum germination temperature of 20°C a result which is somewhat confusing. After viewing these results, and reconsidering those from the experiments on optimum minimum maximum temperatures it is reasonable to suggest that *S. prostrata* seeds will germinate successfully at low temperatures, for example between 5 and 15°C, and that the optimum range for germination is at lower temperatures than suit *S. microphylla.*

4. Discussion

The influences of temperature regimes on the germination of seeds has been extensively studied and/or documented by a number of workers (eg. Thompson 1974b, Bewley and Black 1982, Thompson and Grime 1983, Fenner 1985, Bradbeer 1988). It is unfortunate that the experiments on the optimum minimum and maximum temperatures and germination rate could not have been carried out for a longer period of time as the results of long term experiments would probably show that there are a wider range of temperatures at which good germination percentages can be attained. It is likely that embryo growth is slower at cooler temperatures but not halted altogether. The evidence for this is the low number of seeds which did germinate at these temperatures. Germination at high temperatures consistently fell victim to microbial attack and germination was probably stopped as a result of this rather than as a result of the high temperatures themselves.

It is also possible that germination responses to temperature may differ between individual seeds and within genetic provenances of individual populations. Mayer and Poljakoff-Mayber (1982) state that germination response to temperature is controlled by many factors including endogenous compounds, genetic differences in seeds and even the age of seeds. This is a particularly significant point for both species, but particularly *S. microphylla* which, in so many of its other characteristics (eg. flowering time) has noticeable phenotypic differences between individuals. The fact that seed for these experiments were collected from 5 different individuals probably counteracts this somewhat. This may explain the large scatter of days over which germination takes place, regardless of temperature or light conditions. Genetic differences between seeds may be responsible, or the variability may be the result of an internal mechanism delaying the germination of some seeds longer than others. Gulliver and Heydecker (1972) state that the uniformity of germination is modified by temperature, with the scatter of days over which germination takes place greater at lower temperatures. For *S. microphylla* and *S. prostrata* the greatest scatter of days still occurs when temperatures are optimum for germination. This suggests that there is another factor, influencing the germination times of these seeds.

Temperature fluctuations are one of the most effective ways of limiting germination to gaps in vegetation (Fenner 1985), the other being the filtering effect of leaf canopies on light, operating through the phytochrome system (Bewley and Black 1982). Vegetation acts as a buffer to diurnal temperature change, and when gaps in the vegetation appears the fluctuation in temperatures stimulates germination. The positive response of *S. prostrata* to temperature fluctuations may have been a result of this or it may due to the frequent sudden temperature changes in the exposed environments in which seeds are often located. The negative response of *S. microphylla*, indicated by a drop in germination percentage, may well be a consequence of burial, as soil acts as an insulator against such fluctuations. Therefore, it is possible that *S. microphylla* is more accustomed to germinating at relatively constant temperatures rather than fluctuating ones. However, Thompson Grime and Mason (1977) state that diurnal temperature fluctuations may be a mechanism for starting the germination process in buried seeds at times and places propitious for seedling establishment. The influences of fluctuating temperatures are logically greater near the surface of the soil and then decrease rapidly with depth (Thompson and Grime 1983) and therefore the depth at which seeds were germinating from would have a crucial bearing on the influence of temperature fluctuation. Many *S. microphylla* seeds are probably buried deep in the profile before germination takes place and are therefore less exposed to the effects of temperature fluctuation. Contrastingly, it is conceivable that many *S. prostrata* seeds germinate after only shallow burial, which would therefore make them more prone to the effects of temperature fluctuation.

The experiments on seed burial suggest that the seeds of both species are adapted well to germinating after burial in the soil. The apparent ability of both *S. microphylla* and *S. prostrata* to germinate well in the dark even at different temperatures, coupled with the poor establishment of seeds germinated on the surface of the soil supports this. The fact that many of the best germination percentages were achieved after burial of the seeds at depths of 50mm suggests that the significance of light is probably very low indeed, especially considering that a 2mm layer of sand permits less than 2% of light to penetrate (Bewley and Black 1982). However, it must be

remembered that the seeds of both *S. prostrata* and *S. microphylla* generally produced good germination percentages in all light conditions, even full sunlight. Thus, the seeds of these species would probably be described as non-photoblastic, ie. light is neither essential (positively photoblastic) during germination nor does it inhibit (negatively photoblastic) germination (a concept described by Bradbeer 1988). The apparent non-sensitivity of *S microphylla* and *S. prostrata* seeds appears to be characteristic of larger seeded species (Thompson and Grime 1983).

It is probably safe to conclude that germinated seedlings would compete well in poor light environments if only light and shoots from germinating seeds were involved. Both species undergo etiolation when germinated in the dark. That is, they have a tall weak straggling stem, very small unexpanded foliage which is yellowish white in colour. Etiolation is an important adaptation and is present in a number of species. It allows germinating seedlings to elevate their photosynthetic organs above the soil, leaf litter and shading vegetation to reach a level of irradiation sufficient to maintain the seedling in photosynthetic autonomy (Bradbeer 1988). The ability of all seedlings to maintain themselves in a healthy condition even in the presence of very shaded conditions shows conclusively that the seedlings of both species are shade tolerant. Since it is likely that *S. microphylla* originally existed in Canterbury as a forest species and *S. prostrata* seems to germinate regularly under the dense matted canopy of its parents, establishing seedlings of both species are probably exposed to highly shaded environments.

It must be remembered that no attempt was made here to experiment with root competitive effects. They create another array of conditions which may affect seedling growth.

5. Summary

In summary, it would appear that in broadly speaking the seeds of both *S. microphylla* and *S. prostrata* are sensitive to temperature but have marginal if any sensitivity to light when germinating. Both species have an optimum germination temperature range of approximately 10-20oC. During germination it appears that temperature fluctuations also have an influence,

improving the germination percentages of *S. prostrata* and apparently decreasing them for *S. microphylla*. After germination has taken place the early growth of new seedlings is influenced by light, particularly those of *S. microphylla*, with height growth more substantial in poorer light environments. Both species display etiolation. Most seeds of both species will germinate from the soil in good numbers if sown at depths of 50mm or less. From very deep in the profile, in a zone where oxygen content is questionable, a small percentage of *S. microphylla* seeds will still emerge reinforcing its ability and preference to germinate from this locality.

CHAPTER 8

OVERCOMING SEED COAT IMPERMEABILITY IN NATURE

1. General Introduction

It is well known that for the majority of *S. microphylla* and *S. prostrata* seeds, scarification (breaking the integrity of the seed coat) is required before germination can take place. It is not well understood how *S. microphylla* and *S. prostrata* seeds become scarified in nature. It is generally accepted that the presence of the tough testa is a mechanism of dormancy, delaying the onset of germination. There are a number of natural mechanisms which may be responsible for scarification, and 3 main ones have been studied in this chapter. They are: abrasion in riverbed systems; fire and microbial activity. These are considered to be the 3 most likely mechanisms for seed scarification of these particular species, although there may be others responsible.

Fire, as already mentioned, has been predominant in the history of the Canterbury landscape and is probably something which has affected both species and their seeds for a long time. It is thought that the action of grassland and scrub fires would be of particular importance as the frequency of fires of this nature, on the Plains especially, seems to have been the greatest. The large numbers of seeds that lay on the surface of the ground for both *S. microphylla* and *S. prostrata* must have been prone to the effects of these fires. However, the role of humus fires has also been considered in this chapter for *S. microphylla*, as this may give an indication of the effects of fire on seeds which have fallen in forest environments or been buried at shallow depths. As *S. prostrata* does not grow in forest environments the effects of humus fires have not been studied for this species. Germination of seeds has also been studied to assess the destructive effects fire may have on the growth of the embryos. Despite the obvious effects fire may have on seeds, many of them, particularly those of *S. microphylla*, end up deeply buried. These seeds may escape the actions of fire, so long as they were buried at sufficient depth.

With rivers moving across the plains and wind now playing a larger role in the dispersal of *S. microphylla* seeds it is possible that large numbers of seeds find their way into river systems. The actions of large braided rivers such as those on the Canterbury Plains, which carry large quantities of sediment, must be one of abrasion. A crude riverbed simulation experiment has been done to investigate whether this activity could possibly scarify the seeds. This experiment has only been conducted on *S. microphylla* as water systems have a minor if not non-existent role on the seed ecology of *S. prostrata*.

2. Methods

(a) Fire

Two metal trays approximately $1m^2$ in area, and 4cm deep were filled with soil to act as the ground. On tray 1 a grassland fire was simulated and on tray 2 a humus fire. On the surface of the soil in tray 1 100 *S. microphylla* and 30 *S. prostrata* seeds were scattered, while only 100 *S. microphylla* seeds were scattered over the surface of tray 2. An artificial grassland was generated by collecting large amounts of dry grass and spreading it over the seeds in tray 1 to a depth of approximately 30cm. Similarly, to simulate a humus environment litter from indigenous forest was collected and placed over the seeds in the other at a depth of approximately 4cm. This fuel was then ignited and burned until completion. Seeds were then removed from the surface of the soil in each tray with tweezers and washed to remove any excess dirt or ash. The 100 *S. microphylla* seeds from each tray were divided into 4 lots of 25, and transferred to separate germination pads in 9cm sterilised petri dishes along with the 30 *S. prostrata* seeds. A control of 100 *S. microphylla* seeds and 30 *S. prostrata* seeds which had not been burned were also sown on germination pads. The petri dishes were then placed in a glasshouse, kept at a uniform 20°C, with ordinary daylight as the light source. Dishes were kept moist but not too wet and monitored daily for imbibition and at 3 daily intervals for germination. The duration of the experiment was 30 days.

(b) Riverbed simulation

It must be noted first of all that there are many variables controlling the movement of seeds in a riverbed system; the speed and volume of waterflow, the size and quantity of sediment and the buoyancy of seeds etc. These things also vary throughout the course of the river. Therefore this experiment cannot fully simulate a river system as such, but may show whether it is possible for the actions of sediments and water in a river to scarify *S. microphylla* seeds to the point of imbibition.

To do this, 100 *S. microphylla* seeds were collected and gently shaken in a mechanical shaker, with a mixture of sediment and water for a period of 60 minutes. The sediment was collected from the riverbed of the Waimakariri river and varied in grade from fines (less than 2mm) to large stones (approximately 200mm). After 60 minutes the sediment was spread out on a metal sink bench, and the seeds separated and washed. The number of seeds with obvious chips and cracks in the seed coat were then recorded. Seeds for which this was not obvious were placed on germination pads and put in a glasshouse for several days under the same conditions as those described for fire to see if any imbibition would occur. Because the intention of this experiment was only to study seed coat scarification in *S. microphylla*, germination experiments were not done.

(c) Microbial activity

A simple experiment was done to see if there was any direct effect of microbial activity in the soil. In small germination trays 100 seeds of *S. microphylla* and 30 of *S. prostrata* were sown at depths of 30mm in soil (bio-blend). The soil was kept moist, but not wet and the trays placed in a glasshouse maintained at a uniform temperature of 20°C. After 90 days the seeds were excavated from the soil and examined under a microscope for any signs of microbial growth.

3. Results

(a) Fire

The magnitude of imbibition for both *S. microphylla* and *S. prostrata* after being exposed to a both types of fire was significant. For *S. microphylla*, a total imbibition of 66% (error 0.66+/- 0.0476) and 18% (0.18+/- 0.0386) was recorded under the grassland fire and humus fire respectively. In effect, this means that 66% and 18% of seed coats under each respective fire regime, were damaged by fire to the point of allowing imbibition to take place. In the control 0% of seeds imbibed (as expected). Contrastingly, no germination was recorded at all over 30 days for imbibed seeds of either the fire regime or the control.

The results for *S. prostrata* were very similar. After being exposed to the grassland fire 73.3% (0.733+/- 0.0822) of seeds imbibed, but only 20% (0.2+/- 0.0743) germinated. This suggests that fire is very destructive to the germination of both species even though imbibition takes place. The inability the seeds of both species to deal with fire in this way is surprising, especially considering how integral it has been in the vegetation history of Canterbury. It must be noted however, that although large numbers of seeds on the surface of the soil in *S. microphylla* stands may be destroyed by fire, there are much greater numbers beneath the soil which would escape the effects. Because the seeds of *S. prostrata* do not have extensive seed banks, logic would suggest that their seeds would be more prone to the effects of fire. This may be the reason why some of their seeds did germinate after exposure. There was a very long elapsed time between exposure to the fire and germination for those seeds of *S. prostrata* which did germinate. The reason for this is not clear except that the action of fire on the seeds may cause internal behaviour of the seeds to alter, slowing germination.

(b) Riverbed simulation

After seeds had been shaken with the alluvium mix, it was found that it had a profound effect. Of the 100 *S. microphylla* seeds that had been used for experiment, 48% (0.48+/- 0.0502) had been totally destroyed. Only fragments of these seeds (mainly seed coat material) were found. Of the 52 remaining seeds 43 (43% overall: 0.43+/- 0.0498) had obvious damage to their seed coats, and many of them were already showing signs of imbibition. Only 3 seeds (3% overall: 0.03+/- 0.0174) imbibed out of the remaining 9 seeds after being placed in the glasshouse. There was no imbibition in the 9 control seeds.

In effect this means that in total, 94% (0.94+/- 0.0245) of seeds were either damaged to the point of potential imbibition, or destroyed. It is difficult to believe that a riverbed unless very turbulent and carrying a lot of sediment, would have this kind of effect on the seeds. Because of this, it is considered that this simulation is perhaps more severe than was anticipated. Nevertheless, it does illustrate that a riverbed may indeed have the potential to scarify seeds mechanically and that the destruction of some seeds in riverbed systems may also occur.

(c) Microbial activity

In both *S. microphylla* and *S. prostrata* there was no evidence of microbial decomposition of the seed coats in the soil. No change in the seed coats of either species was observed after 90 days. However, 6 (20%; 0.20+/-0.0-743) *S. prostrata* seeds did germinate, but the absence of microbial activity on the other seeds indicated that this was not a result of microbial decomposition of the seed coat. This is an important finding, and suggests that the seed coats of *S. prostrata* are more penetrable than first thought. It appears that the seeds which germinate are young mature seeds, which suggests that the seed coats of *S. prostrata* harden after the onset of seed maturity.

Laboratory experiments on microbial decomposition were conducted on both the whole and ground seeds of *S. microphylla* and *S. prostrata* by Dr L Greenfield (Department of Plant and Microbial Sciences, University of Canterbury). He exposed the seeds in each respective state to microbial decomposition at 20°C and optimum moisture for 35 days. There was no microbial growth at all on the whole seeds of either species over the 35 day period. The weight loss (ash free) of *S. microphylla* was only 8% while there was no weight loss at all for *S. prostrata*. Contrastingly there was substantial microbial growth on the ground seeds of both species and weight losses of 51% and 46% for *S. microphylla* and *S. prostrata* seeds respectively. This seems to show conclusively that the whole seeds of both species, with their seed coats intact, are extremely resistant to microbial growth in the short term. It is only when the interior contents of the seed are exposed that microbial activity initiates. This may explain why the artificially chipped seeds used in the majority of experimentation in this project have been so prone to microbial attack while other unscarified seeds were not. From the natural standpoint Dr Greenfields findings have shown that microbial decomposition of intact seeds in the soil would probably be rare, if not non existent. Despite this the question still remains as to how the seeds become permeable in nature. Dr Greenfields experiments have not addressed the effects of microbial activity over a very long period of time which, with the influences of other soil factors, may cause some decay.

4. Discussion

The first important finding from this study is that there is a small percentage of *S. prostrata* seeds which will germinate without scarification. It was assumed at the beginning of this project that scarification was a necessary prerequisite to the germination of *S. prostrata* seeds. This finding prompted basic germination trials on *S. prostrata* seeds to determine the actual germination percentages of mature unscarified seeds under optimum conditions. The results showed that germination without scarification occurs at about the same percentage as seen in the soil/microbial experiment (30%: 0.3 +/- 0.0851). As stated earlier, the seed used in experimentation for this project were a mixture of ages and on closer inspection it was found that it was only seeds taken

from younger seed pods which had germinated. The most probable explanation for this is that the seeds in these pods had not yet reached full maturity but had germinated, displaying similar behaviour to immature *S. microphylla* seed. However, some of the pods from which unscarified *S. prostrata* seed germinated had already been lost from the plant suggesting that full maturity had been reached. If full maturity as recognised by the plant was reached, it would suggest that hardening of the seed coat generally occurs after seed fall not before, unlike *S. microphylla*. Unfortunately this was not able to be substantiated. The ability of some *S. prostrata* seed to germinate without scarification may well be a mechanism to avert the necessary action of scarification in some seeds. The soil in some areas which the seeds may fall may be poor and shallow, and therefore unable to bury *S. prostrata* seeds successfully. If influences in the soil after burial were necessary for the scarification of seed coats, as it appears to be for *S. microphylla*, then these seeds would fail to germinate. Likewise, there is a distinct migration of *S. prostrata* seeds downslope under the action of gravity. It is possible that a lot of seeds are lost in this way, ending up in forested systems to which they are not suited, or in rivers where they also fail to germinate. If some seeds were able to imbibe and germinate soon after their release, then establishment of roots in transit may stop this movement.

Both species were sensitive to fire although *S. microphylla* seems to be more affected than *S. prostrata*. It is fortunate that *S. microphylla* has extensive soil seed banks or the fires that became so prolific during the Polynesian era may have been detrimental to the species. Although some seeds appear to survive after fire it is likely that none will germinate. The sensitivity displayed by both species to fire is also surprising when considering the ecologies of many close relatives like Gorse (*Ulex europeus*) for example, which proliferates after fire.

The majority of seed coat breakdown in *S. microphylla* appears to occur in the soil. Initially it was suspected that seed coat breakdown in the soil was attributable to microbial decomposition of the seed coat, due to the prolific microbial activity seen during experimentation. The same was thought of *S. prostrata*. The discovery that whole seeds of both species are particularly resistant to microbial activity, at least in the short term, shows that some other mechanism is responsible.

There are three things which may cause the resistance of these seeds to microbial breakdown. Either the concentration of toxic chemicals in the seed coats are too high to facilitate microbial growth or the osmotic potential created by these chemicals prevents microbial penetration (Greenfield 1992 unpubl) or the seed coat is constructed from a very resistant organic material (Burrows 1993 pers comm). The rapid and apparently unimpaired growth of microbes once the seeds had been ground suggests that the barrier to microbial growth lies in the seed coat and that microbial organisms are particularly partial to feeding on the interior contents of these seeds. This poses a possible problem for *S. microphylla* and *S. prostrata* in nature since microbial destruction of seeds may occur once they have become scarified and the surrounding elements are able to invade the interior of the seed. This may explain why, after scarification, some seeds of both species have very rapid germination (some in as little as 3 days after imbibition). Selection for this speedy germination would have occurred through evolutionary time.

With the increased role of wind in today's environment through the loss of surrounding vegetation, it is likely that more *S. microphylla* seeds particularly, are finding their way into river systems. The destructive nature of the riverbed simulation experiment seems to show that a number of seeds may actually be destroyed in these systems. This may explain why some *S. microphylla* seeds are buoyant. Buoyant seeds would be less prone to the destructive effects of an active riverbed. The apparent non-buoyancy of *S. prostrata* seeds suggests that they are not suited to long distance dispersal in rivers and most of their viable seeds would probably be lost in river systems. This reinforces the view that *S. prostrata* seeds probably rely much more on terrestrial dispersal mechanisms.

5. Summary

In summary, there is breakdown of *S. microphylla* seed coats in the soil, the evidence of which was the discovery of germinating seeds in soil profiles. However, it is still difficult to say conclusively what the causal mechanism of this breakdown is. Although abrasion in the soil may be a possibility, it is highly unlikely due to the exceedingly slow movement of seeds and soil

particles in the profile. It is hard to imagine that such movement would create the necessary scarification forces. The key to understanding how *Sophora* seeds become permeable in the soil may be to investigate what happens at the micropyle.

The action of fire and riverbed systems can be detrimental to seeds although this does not refute the fact that *S. microphylla* uses water as a long distance dispersal mechanism. The fact that some seeds are buoyant may be an adaptation to aid this.

Older *S. prostrata* seeds probably undergo a similar abrasion process if assimilated into the soil, as their seed coats appear to be tough and impenetrable. Some seeds will germinate after exposure to fire although the vast majority appear to be destroyed. Alternatively younger seeds, if released from their seed pods, may be able to germinate immediately if conditions are favourable. If they are not favourable and they fail to germinate the seed coats become hard and dormancy takes place in a similar fashion to *S. microphylla* until scarification occurs and releases the seeds.

CHAPTER 9

DISCUSSION

1. General Introduction

In this chapter my findings on the seed ecology of *S. microphylla* and *S. prostrata* are summarised in relation to findings of other workers on these and other legumes with hard coated seeds. It also gives a brief summary of my results highlighting new discoveries in the light of other work done on *S. microphylla* and *S. prostrata.* The chapter is divided into three parts: general discussion; flow diagrams of seed fate in *S. microphylla* and *S. prostrata* and finally future directions that research on these species might take.

2. General discussion

In Canterbury today *Sophora microphylla* is common on terrace country along major rivers, giving the impression that it thrives in exposed habitats. Although it is likely that it has always grown successfully in open sites, *S. microphylla* probably also lived as a forest plant in pre Polynesian times, inhabiting the kanuka stands or podocarp-broadleaf forest which once covered most of the Canterbury Plains. Forest dwelling *S. microphylla* can still be seen on Banks Peninsula.

The arrival of the Polynesians and then subsequently the Europeans saw the destruction and failure of most of the forest species on the Plains and their replacement first by short tussock grasslands and then (after 1850) by introduced grassland ecosystems. Despite this, *Sophora microphylla* has continued to persist on the Plains, particularly on lower river terraces, where the trees are regarded as useful stock shelter on farms. The survival of *S. microphylla* suggests a versatile ecology, displaying the ability to adapt and survive in a variety of environments.

Remaining populations still manage to produce large quantities of seeds although since there are few juvenile plants evident, it does not appear to be regenerating vigorously. Apparently, the actions of farm machinery and grazing stock often destroy establishing seedlings. Since the vast majority of land on the Canterbury Plains is now subject to this kind of activity there are few remaining sites suitable for establishment of *Sophora microphylla*. Despite this, the prolific natural populations along the banks of Canterbury rivers suggests that some seeds find their way to suitable establishment sites from time to time.

As outlined in Chapter 1, the majority of studies on the biology of kowhais have been conducted by Godley and his collaborators and the main thrust of these studies has been on seed dispersal. Godley's 1982 paper on the common kowhai (*S. microphylla*) however, described the developmental phases of the pods and seeds, showing that there are three distinct phases to fruit maturation; elongation of the seed pod, growth of the seeds and drying and dispersal. In the present study, knowledge of *S. microphylla* seed has been advanced by applying time frames to the developmental phases of the seed and establishing that the times taken for ripening and embryo maturity are distinctly different. Embryos may mature and germinate in as little as 14 weeks while seed ripening, manifest in the drying out of the seed contents and hardening of the coat, takes approximately 20 weeks. This means *S. microphylla* seeds display an ability to germinate when immature and if large quantities of seed are lost when they are young due to a catastrophic event (for example a gale force wind) then it is possible that some may still germinate. Although germination can take place as little as 14 weeks from pollination, seeds are not usually shed from the tree when they are immature.

Although *S. prostrata* has not received the same emphasis in this project as *S. microphylla*, some interesting features of its seed ecology have been revealed.

S. prostrata is a highly branched and low growing shrub, as its name suggests, and occurs in open, exposed sites, where competitors are often lacking. One of the most characteristic features of the sites in which *S. prostrata* grows is the steep slopes that are almost invariably present.

Banks Peninsula has a large number of these types of sites and supports large populations of the plant. However, *S. prostrata* can also be found on the Canterbury Plains in a fairly scattered distribution, often in gullies, or embankments and gorges.

S. prostrata seeds mature in much the same way as *S. microphylla*, although flowering usually occurs slightly later. Consequently, seed maturity can be as late in the year as June. It has fewer seeds per pod than *S. microphylla* and the pods and seeds themselves are small and lighter in construction. One of the main differences in the seeds of the two species is that *S. prostrata* appears to take much longer to harden its testa than *S. microphylla*. New seasons seeds, which appear mature, may still have a soft seed coat and will imbibe without scarification. Many pods are released from the plants when the seeds are in this state.

Young *S. prostrata* seedlings are prone to similar grazing pressures on Banks Peninsula as *S. microphylla* is on the Canterbury Plains. However, the often inaccessible sites on which the plant grows probably means that some establish without problems from grazing stock. Also, it would appear that the adults at least are unpalatable to stock, with many of the mature individuals at Dyers Pass (Port Hills) showing no signs of browsing. The establishment of seedlings is likely to be one of the biggest problems facing *S. prostrata*. The rough sites and often steep slopes means that many seeds move down slope rapidly under the influence of gravity. It seems that in this situation the plant relies on there either being suitable sites downslope to 'trap' seeds and facilitate germination, or assimilation into the soil, or being 'trapped' beneath the dense matted canopies of the parents and germinating from there. Some seeds are lost into waterways and forest and in general this would probably mean the death of these seeds. There is no doubt that the dense clump's of *S. prostrata* found on Banks Peninsula consist of more than one individual which suggests that germination from beneath parent plants is characteristic behaviour. Research on the genetics of individuals over an altitude gradient may establish whether the movement of *S. prostrata* downslope is an important part of its ecology. Interestingly, establishment tends to be most successful after seeds have been buried; a similar characteristic to *S. microphylla*. This was

contrary to what was expected. It was thought that with a relatively difficult establishment phase seeds would establish more successfully on the surface of the soil, not beneath it.

The main way in which *S. microphylla* seeds were lost was through predation by the larvae of the moth *Stathmopoda aposema* and they accounted for approximately 28% of seeds in my year of study. No work on the predation of *S. microphylla* seeds was found in the literature, although the taxa and predatory nature of *Stathmopoda aposema* was well known to several New Zealand entomology experts (eg John Dugdale). Some recent work has been done on the development of *S. aposema* by Dr J R Clearwater and Mrs S. J. Marsh of Horticultural research Auckland New Zealand, although no published results have been seen to date. Thus, all of the work documented in this project including the amount and magnitude of predation, mechanisms of infestation, and methods of predation are probably original and have provided insight, particularly into the annual magnitude of seed loss sustained by *S. microphylla.* Although some *S. prostrata* seeds are lost to predation, the proportion seems much lower than in *S. microphylla.* It is possible that the lack of predated seeds may be a result of the much lower seed production in this species. Although specific statistics for the proportion of predated seeds was not determined for *S. prostrata*, very few predated seeds were found, leading me to conclude that the absence of predation in this species is more likely to be a consequence of the environment and altitude from which the seeds were collected rather than the amount of seed. The site is consistently more windy, moist and cool than the Plains and adult *Stathmopoda aposema* moths may find these conditions unfavourable.

Although information on the predation of *Sophora* seeds is poor, this is not the case for other related legume species. Apart from those of agronomic importance (peas, beans etc) seed predation has been well documented for *Acacia* in particular. According to New (1983) much of the earlier knowledge of *Acacia* seed mortality due to insects has been based on the extensive survey by Janzen (1975, 1980) on Central American species which are often attacked by Bruchid beetle larvae. On reviewing Janzen's work it was found that, as with *Stathmopoda aposema*, these insects also accounted for the loss of large proportions of viable *Acacia* seeds, up to 70% in

some cases. In Australian studies of seed predation on *Acacia* species, larval and adult weevils (*Melanterius* spp.) are the principal seed predators (Auld 1983) although there are a wide variety of others. Interestingly, although there appears to be a wide array of different insects that predate *Acacia* (and other leguminous seeds in general) they have a similar method of infestation and predation to *Stathmopoda aposema*, and this is particularly true of weevils. Auld (1983) and New (1983) document features of the predation of weevils on Australian legumes which reveal striking parallels between them and the effects of *S. aposema* in *Sophora*, most notably in the method of eating the seeds and pupation. This suggests that the way in which leguminous seeds are predated by insects is more a result of the physiology of the seeds, rather than the peculiarities of the insect predators themselves.

Most *S. microphylla* seeds are dispersed locally by the actions of either gravity or wind. Seed dispersal, particularly over long distances in *S. microphylla*, has been documented extensively by Godley et al, (eg Sykes and Godley (1968), Markham and Godley (1972)) although the amount and annual distribution of seed fall has not been addressed by Godley or other workers. The accounts of dispersal given in this project, particularly local dispersal, amplifies their work.

Long distance dispersal of *S. microphylla* seeds is undoubtedly achieved by transport in water. The buoyancy displayed in up to 27% of seeds (Sykes and Godley 1968) shows that *S. microphylla* has adapted to dispersal in this way and probably aids the long distance dispersal process of some seeds. Sykes and Godley (1968) attribute this buoyancy to the lower density of the embryo. Although documentation of this buoyancy characteristic could not be found for other legume seeds in the literature, there is substantial evidence to suggest that long distance dispersal by water is a characteristic feature of some legume species. For example Stevens and Hughs (1973) state that water is a primary dispersal agent of adventive broom (*Cytisus scoparius*) in New Zealand, although upstream and uphill spread were said to be caused by projection from bursting pods and seeds carried on the exterior of animals. In Canterbury particularly, the downstream colonisation of riverbeds by lupins (*Lupinus* spp) is extensive in some areas (pers obs) displaying the effective use of water as a dispersal medium. It is possible that the hard seed

coat seen on so many leguminous species is an adaptation to cope with this kind of dispersal. It is probable that these other leguminous species undergo similar scarification and dispersal processes in the riverbed systems.

S. prostrata is simpler in its dispersal mechanisms and seems to rely solely on the actions of wind to disperse its seeds although gravity has an obvious role in hilly environments. The lighter construction of the pod and seeds have probably been selected through evolutionary time for dispersal by wind. Wind appears to have an important role in the dispersal of the seeds of both *S. microphylla* and *S. prostrata* and would probably be important in the dispersal process of most other legumes which produce pods. Dispersal by wind of the whole pods and individual seeds as seen in *S. microphylla* and *S. prostrata* would occur in a similar fashion in these other species.

In many other related legume species consumption of the seeds by birds or transport of the seeds by insects are primary dispersal mechanisms. For example Australian *Acacia* species often use birds such as parrots and pigeons to disperse seeds and ants sometimes carry them significant distances from parent trees (O'Dowd and Malcolm Gill 1986). Ant dispersed species have an attachment on their seeds called a caruncle (or elaiosome-oil body) which is attractive to ants as food. Consequently they collect and transport the seed and consume the caruncle; but the seed remains undamaged and viable. Ants probably fail to consume the rest of the seed because of thick durable seed coats (O'Dowd and Malcolm Gill 1986). *Luzula* spp. and *Astelia* spp. in New Zealand possess a caruncle (pers comm C. J. Burrows) but it is not known whether they are ant dispersed.

The use of animals and insects to disperse seeds in other leguminous species is in complete contrast to *Sophora microphylla* and *S. prostrata* which have little known involvement with animals during their dispersal phase. The only reference to birds or animals eating kowhai seed found was by Potts (1870) who observed kakas eating the seed pods. Kakas usually crush the seeds that they eat but some hard coated seeds such as those found in *S. microphylla* may survive. This may explain how some very remote populations of *S. microphylla*, particularly in

the South Island mountain country, came into existence (ie. through dispersal by kakas). It is possible that moas also ate the pods and dispersed the seeds. The hard seed coats may have helped to preserve some seeds in the gizzards of these birds. However Burrows (1993 pers comm) after looking at approximately 25 moa gizzards has found no trace of kowhai seeds.

The tree seed bank, or the presence of large numbers of old seed pods, is a characteristic feature of *S. microphylla* individuals and has been noted as occurring in other legume species for example black wattle (*Acacia mearnsii*) (Sherry 1971). The ecological reason why seeds are retained on the trees for up to two years remains obscure, although presumably there is some selective reason for it to occur. Various other species in other families also bear considerable seed banks on the plant eg. *Leptospermum scoparium*, *Eucalyptus* spp (Myrtaceae), *Banksia* and *Hakea* (Protaceae) (Burrows 1993 unpubl). In Australia this is recognised as a strategy to enable the plants to release seeds after a fire and this might also apply to *Sophora*. It seems that the amount of retention on *S. microphylla* is largely a result of environmental influences, especially wind. The more frequent and strong the wind is, the more seed pods or individual seeds that will be dislodged from the tree.

Extensive soil seed banks of *S. microphylla* and longevity of seeds is not uncommon among legumes either. There appear to have been no comprehensive studies done specifically on the soil seed banks of *S. microphylla*. Partridge (1989) did study the soil seed banks of secondary vegetation on Banks Peninsula and noted that *S. microphylla* seeds often exist in a viable state even after deep burial. Other related species for example gorse (*Ulex europaeus*) (Ivens 1978, Zabkiewicz and Gaskin 1978) and broom (*Cytisus scoparius*) (Stevens and Hughes 1973) also have large, long lived seed reservoirs in the soil. Ivens (1978) stated that gorse seeds may live for up to 26 years in the soil. A feature of the seed banks of *S. microphylla* that was examined in the present study was the comparative absence of seeds below 10cm in the profile. This suggests that perhaps most seeds germinate before they reach 10cm in depth, or that the seeds seldom ever become more deeply buried in normal circumstances. There seems to be little doubt that burial deeper than this will compromise their germination and emergence ability.

It is evident that there are a range of germination requirements for *S. microphylla* and *S. prostrata* seeds. The most obvious of these is breakage of the seed coat but there are a variety of others. The most apparent of these, from this study, is the sensitivity of germinating seeds to temperature. Although some seeds will germinate at temperatures ranging from 5-30°C, there seems to be a distinct optimum at 10-20°C for *S. microphylla*. The optimum temperature range for germination of *S. prostrata* seeds is about the same as for *S. microphylla* (10-20°C), although there is a better germination response at lower temperatures, and a poorer response at higher temperatures. Seedlings of *S. prostrata* seem to possess a similar ecology to those of *S. microphylla* with distinct etiolation and shade tolerance. Shade tolerance and etiolation would be advantageous to *S. prostrata*. In pre-Polynesian times the proliferation of large tussocks and woody shrubs in open environments probably meant that seeds would be germinating in deep shade. Such adaptations would also be an advantage in view of the apparent propensity *S. prostrata* seedlings have for germinating under the canopies of their parents. As might be expected there was no conclusive evidence that germinating seeds of either species were sensitive to light. It is thought that due to the very thick testa on the seed, and because most seeds probably germinate after burial in the soil, the role of light would be fairly insignificant. The marked success of germination after the burial of scarified seeds, and more importantly the apparent establishment failures of seeds germinated on the surface of the soil, suggests that the seeds have a preference for germination after burial. Further evidence for this is the distinct etiolation of seedlings in an effort to reach light, a mechanism to achieve emergence after deep burial of up to 10cm. Etiolation and emergence after deep burial suggests that *Sophora* seedlings are well adapted to germinating from within the soil. However, etiolation may not necessarily be a beneficial adaptation, especially in the long term. Ivens (1978) in a study of some of the aspects of the seed ecology of gorse (*Ulex europaeus*) noted that gorse seedlings also etiolated when germinating. Etiolation was noted as a possible cause of fungal attack in young seedlings because they were weak and under stress. From this, it would appear that although etiolation may be beneficial to *Sophora* in the short term there may be detrimental consequences in the long term if seedlings fail to reach light. One of the most outstanding features of *S. microphylla* and *S.*

prostrata to come out of the experiments on light is the apparent shade tolerance of both species. It is generally considered that legumes are pioneering species and would therefore have relatively high light requirement during germination and early growth. Shade tolerance in legumes does not seem to have been documented much in the literature to date although Brokaw (1985) stated that the legumes of tropical forests have obligate requirements for gaps during germination and early growth and many are emergent trees when the gaps close.

Studies on the germination characteristics of seeds, particularly those concerning the influences of temperature and light are extensive for many other species, legumes and non-legumes alike but to date none have been documented for either *S. microphylla* or *S. prostrata.* The most important discovery to come out of my research was the marked sensitivity to temperature and the optimum temperature ranges for germination of the two species. Temperature characteristics seem to be very important in hard coated seeds like *Sophora* and more future research should be directed towards the effects of temperature regimes on the physiology of these seeds. The reason for this is that the best known releases from dormancy in nature of hard coated seeds seems to be associated with the actions of temperature and temperature fluctuations (Bewley and Black 1982). An example of this is the way in which temperature fluctuations cause the strophiole in the seed coat of lupin seeds (*Lupinus* spp.) to crack allowing water entry (Bewley and Black (1982). Further investigation of the influences of temperature on *Sophora* seeds may reveal that temperature has as much, if not more of a key role than mechanical breakdown in the scarification of their seed coats in nature.

Although it is common knowledge that the seed coats of *S. microphylla* have to be broken before germination can take place, no comprehensive studies of the possible mechanisms by which this breakdown takes place in nature had been done prior to this study. Although germinating seeds in the soil seed bank were discovered the actual mechanism of how the breakdown occurs in dormant seeds remains unclear. As mentioned earlier, it is possible that temperature fluctuation is responsible for rendering permeability, which has been proven in other legumes for example lupins. Another possibility is the slow gradual decay of the seed coat, possibly in the micropylar

region. However, it was established that short term decay of the seed coat by microbial activity was not a viable cause of scarification due to the apparent resistance of the seed coat to microbes (Greenfield 1992 unpubl). Some more experiments are needed on kowhais to examine these matters. Other workers have documented a number of artificial treatments which are employed to scarify hard coated seeds (mainly legumes) before experimentation in the laboratory. Sherry (1971) for example described a very long and detailed process involving water at different temperatures to achieve optimum germination in black wattle (*Acacia mearnsii*). In commercial forestry operations acid treatment is well known as a means for making the seed coat of hard coated species permeable to water. Mechanical scarifiers are used for this purpose also eg. with *Robinia pseudoacacia* (Forest Service, U.S. Dept. of Agriculture 1948).

The main characteristic relating to the impermeability of the seed coat in *S. prostrata* which has apparently not been noted before, was the ability of apparently younger mature seeds (some already dispersed) to germinate without scarification. This counters the view that mature *S. prostrata* seeds require scarification before germination as in *S. microphylla* seeds. This feature contrasts with the generally impermeable nature of many legume seeds and it is thought that it is a mechanism to facilitate rapid germination. However, it must be noted that the permeability is only temporary and many seeds will eventually develop a very hard seed coat if conditions conducive to germination do not arise.

The complete failure of *S. microphylla* seeds and the near complete failure of *S. prostrata* seeds to germinate after exposure to fire was not consistent with what has been found for other leguminous species. Gorse (*Ulex europaeus*), as do other leguminous species in general, seem to benefit from the actions of fire. Sherry (1971) stated that *Acacia mearnsii* seeds were 'stimulated by heat' and resistant to damage by fire. In New Zealand, presumably because of its apparent ability to regenerate successfully and vigorously after fire, a lot of work has been done on the effects of fires on gorse seeds. One of the most comprehensive studies done was by Zabkiewicz and Gaskin (1974). They showed that high numbers of gorse seeds were destroyed by fire with a reduction in the number of seeds in burned trial plots to an average of only 66% of the original

unburned population. However, they also showed that of the seeds not destroyed by fire, virtually all were capable of subsequent germination. This is in complete contrast to the seeds of *Sophora* where virtually all failed to germinate. However, it may be that under natural conditions some *Sophora* seeds will escape the most severe effects of fire, have their coats cracked and germinate. The present project took no account of what happens to *Sophora* seeds which have been shallowly buried. It is feasible that a shallow layer of soil may curb the effects of fire enough to allow scarification to take place but yet not kill the embryo. This would be beneficial to *Sophora* seeds which would not only be scarified, but also be able to establish more successfully, due to the buried nature of the seeds. Further experimentation is required on this.

Ivens (1978) found that moderate numbers of gorse seeds will germinate without the stimulation of burning, with areas which had only been cleared and not burned showing moderate numbers of germinating seeds. Anecdotal evidence suggests a similar phenomenon occurs with *S. microphylla* and *S. tetraptera*, particularly in moist, fertile sites. If germination of *Sophora* seeds does occur after clearing vegetation it may pose clues as to what stimulates germination and how the seed coats are broken in nature (which still remain a mystery). Ivens indicated that the reason for the post clearing flush in gorse seedlings remained obscure. One possibility was the increase in light intensity, although it was also stated that the light was not essential for germination (as with *S. microphylla* and *S. prostrata*). Another reason might be that it exposes seeds to greater fluctuations in temperature which might be expected to stimulate germination.

3. Diagrammatic representations of the seed fate in *S. microphylla* and *S. prostrata*

Figure 19 and Figure 20 give diagrammatic representations of the seed fate in *S. microphylla* and *S. prostrata* respectively. The diagrams provide a summary of the influences and processes involved in the life of the seeds.

Figure 20. A diagrammatic representation of the fate of *Sophora microphylla* seeds.

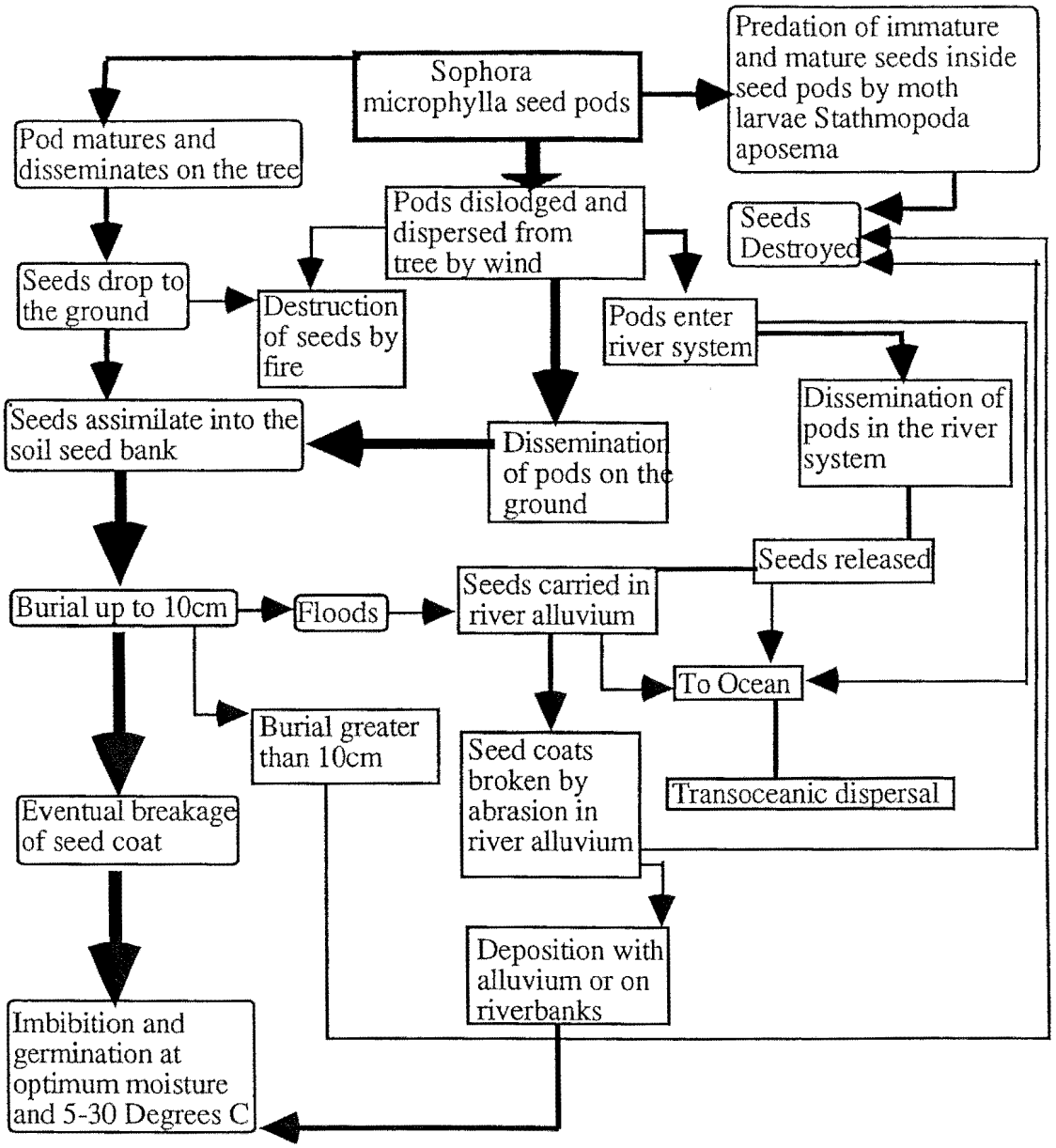
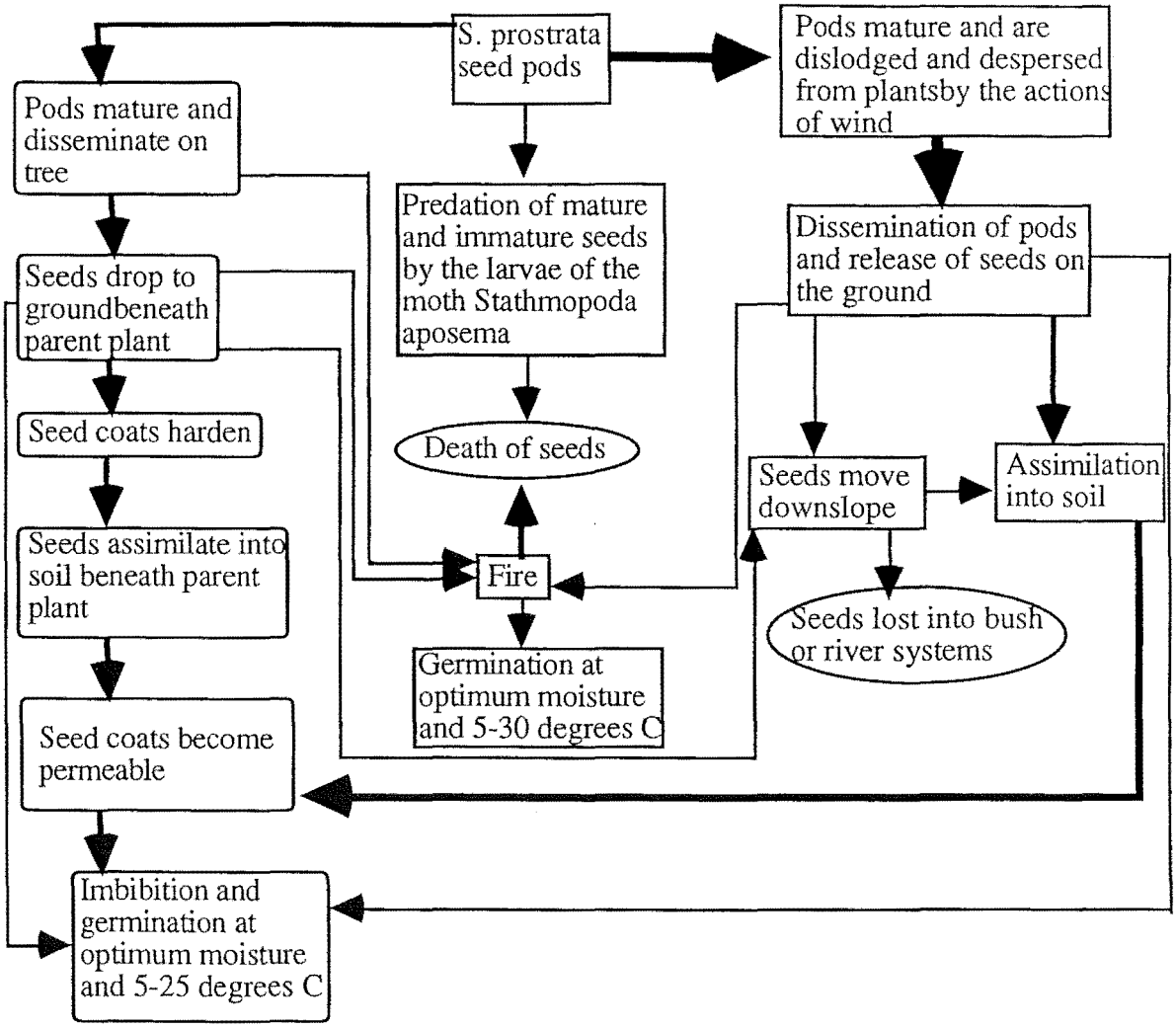


Figure 21. A diagrammatic representation of the fate of *Sophora prostrata* seeds.



4. Future directions

There are still many questions to answer about the seed ecology of *S. microphylla* and *S. prostrata* and this project has only provided a broad investigation into the general aspects of their seed ecology.

The most important area in which research should continue is in the nature of the seed coat. The exact mechanism of how seed coats degrade to the point of imbibition in nature is still not well understood and this is the same for many hard coated species. For *S. microphylla* and *S. prostrata*, research on the influences of temperature on the seed coat, particularly temperature fluctuations, would be useful. As seen above, it has been shown in other legume species that temperature fluctuations are responsible for scarification of the seed coat in nature. They may be a trigger to start germination of *Sophora* seeds in some circumstances. Further research needs to be applied to the long term effects of microbial activity on the seed coat, particularly in the micropylar region. This project has not investigated what happens at the micropyle during germination or after prolonged burial in the soil. Knowledge of the exact composition of the seed coats of *Sophora* species may give an understanding of why they are so resistant to external influences. The reason(s) and significance of why some mature *S. prostrata* seeds germinate without the need of scarification need to be investigated in more depth also.

An in depth investigation into the seed banks of *Sophora microphylla* would be beneficial particularly into the ways in which seeds are assimilated into the soil, the quantity of seeds added to the seed bank in a year, and the age class structure of seeds in the seed bank. This would allow an understanding to be gained of the longevity of these seeds in the soil and their eventual fate. Long term experiments are required to investigate these matters which are of considerable importance to the seed ecology of *S. microphylla* and *S. prostrata*.

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APPENDIX 1

ERRORS

The tables in this Appendix show the standard errors of all the estimated proportions in the text results. The errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Immature germination in *S. microphylla*.(p 33).

Age(wks)	6	8	10	12	14	16	18	20
+/-	---------	---------	---------	0.0200	0.0446	0.0503	0.0502	0.0174

Seed predation. (p42).

Age(wks)	6	8	10	12	14	16	18	20
(+/-)	0.003	0.000	0.006	0.006	0.025	0.021	0.018	0.022

Age(wks)	22	26	30	34	38	42	46
(+/-)	0.022	0.023	0.024	0.025	0.024	0.024	0.024

ERRORS:. These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Seed Fall.

(a) Total pod fall from 5 sample trees.(p56).

Tree 1 +/-	Tree 2 +/-	Tree 3 +/-	Tree 4 +/-	Tree 5 +/-
0.0557	0.0332	0.0743	0.0557	0.0693

(b) Percentage monthly seedfall. (p57).

	Apr	May	Jun	Jul	Aug	Sep	Oct
Tree 1 +/-	0.0920	0.0332	0.0464	----------	----------	0.0743	0.0693
Tree 2 +/-	0.0928	0.0332	0.0557	0.0464	0.0332	0.0557	0.0631
Tree 3 +/-	0.0822	0.0464	----------	----------	----------	0.0743	0.0822
Tree 4 +/-	0.0910	0.0693	0.0557	0.0332	0.0464	0.0332	0.0557
Tree 5 +/-	0.0926	0.0557	0.0743	----------	----------	0.0464	----------

ERRORS:. These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Optimum Minimum Maximum Temperatures. (p71).

Sophora prostrata.

Days	5°C +/-	10°C +/-	15°C +/-	20°C +/-	25°C +/-	30°C +/-	35°C +/-
3	----------	0.0332	0.0332	0.0464	0.0464	----------	----------
6	----------	0.0464	0.0693	0.0743	0.0631	----------	----------
9	----------	0.0557	0.0926	0.0910	0.0743	0.0332	----------
12	0.0464	0.0786	0.0851	0.0822	0.0822	0.0464	----------
15	0.0631	0.0851	0.0786	0.0743	0.0875	0.0464	----------
18	0.0693	0.0895	0.0743	0.0631	0.0875	0.0464	----------
21	0.0743	0.0920	0.0631	0.0464	0.0875	0.0464	----------
24	0.0743	0.0928	0.0631	0.0464	0.0875	0.0464	----------
27	0.0743	0.0928	0.0631	0.0464	0.0875	0.0464	----------
30	0.0743	0.0928	0.0631	0.0464	0.0875	0.0464	----------

ERRORS: These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Optimum Minimum Maximum Temperatures (p71).

Sophora microphylla.

Days	5°C +/-	10°C +/-	15°C +/-	20°C +/-	25°C +/-	30°C +/-	35°C +/-
3	----------	----------	0.0100	----------	0.0142	0.0174	0.0100
6	----------	----------	0.0224	0.0100	0.0245	0.0224	0.0100
9	----------	----------	0.0368	0.0378	0.0327	0.0348	0.0100
12	0.0142	0.0200	0.0409	0.0429	0.0368	0.0378	0.0100
15	0.0200	0.0423	0.0423	0.0469	0.0461	0.0416	0.0100
18	0.0224	0.0473	0.0429	0.0488	0.0461	0.0429	0.0100
21	0.0245	0.0500	0.0441	0.0498	0.0465	0.0435	0.0100
24	0.0245	0.0502	0.0441	0.0502	0.0469	0.0435	0.0100
27	0.0245	0.0498	0.0441	0.0502	0.0469	0.0435	0.0100
30	0.0245	0.0490	0.0441	0.0501	0.0469	0.0435	0.0100

ERRORS:. These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Temperature fluctuation. (p74).

Sophora prostrata.

Time Days	20°C +/-	15/20°C +/-	10/25°C +/-	5/30°C +/-
3	0.0464	----------	0.0464	0.0464
6	0.0743	0.0875	0.0557	0.0631
9	0.0910	0.0875	0.0743	0.0743
12	0.0822	0.0743	0.0822	0.0822
15	0.0743	0.0557	0.0851	0.0822
18	0.0631	0.0557	0.0851	0.0822
21	0.0464	0.0557	0.0851	0.0822
24	0.0464	0.0557	0.0875	0.0822
27	0.0464	0.0557	0.0875	0.0822
30	0.0464	0.0557	0.0875	0.0822

ERRORS:. These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Temperature fluctuation. (p74).

Sophora microphylla.

Days	20°C +/-	15/20°C +/-	10/25°C +/-	5/30°C +/-
3	----------	0.0174	0.0142	0.0100
6	0.0100	0.0402	0.0245	0.0142
9	0.0378	0.0461	0.0327	0.0200
12	0.0429	0.0476	0.0368	0.0200
15	0.0469	0.0476	0.0461	0.0245
18	0.0488	0.0485	0.0461	0.0245
21	0.0498	0.0485	0.0465	0.0245
24	0.0502	0.0488	0.0469	0.0245
27	0.0502	0.0488	0.0469	0.0245
30	0.0501	0.0488	0.0469	0.0245

ERRORS: These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Photoperiodicity. (p76).

Sophora prostrata.

Days	0hrs sunlight +/-	6hrs sunlight +/-	12hrs sunlight +/-	18hrs sunlight +/-
3	0.0631	----------	0.0464	----------
6	0.0786	0.0743	0.0743	0.0332
9	0.0910	0.0822	0.0910	0.0631
12	0.0920	0.0875	0.0822	0.0926
15	0.0743	0.0926	0.0743	0.0875
18	0.0577	0.0910	0.0631	0.0822
21	0.0464	0.0895	0.0464	0.0822
24	0.0464	0.0895	0.0464	0.0822
27	0.0464	0.0895	0.0464	0.0822
30	0.0464	0.0895	0.0464	0.0822

ERRORS: These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Photoperiodicity. (p76).

Sophora microphylla.

Days	0hrs sunlight +/-	6hrs sunlight +/-	12hrs sunlight +/-	18hrs sunlight +/-
3	----------	----------	----------	----------
6	0.0200	0.0283	0.0100	----------
9	0.0348	0.0423	0.0378	0.0174
12	0.0435	0.0488	0.0429	0.0265
15	0.0465	0.0479	0.0469	0.0327
18	0.0501	0.0456	0.0488	0.0338
21	0.0502	0.0435	0.0498	0.0359
24	0.0500	0.0435	0.0502	0.0386
27	0.0499	0.0423	0.0502	0.0402
30	0.0496	0.0416	0.0501	0.0409

ERRORS: These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Germination response of seeds to shading regimes. (p81).

Shade regime	*S. microphylla* +/-	*S. prostrata* +/-
Darkness	0.0496	0.0875
Shade 3	0.0200	0.0557
Shade 2	0.0476	0.0926
Shade 1	0.0490	0.0910
Daylight	0.0402	0.0926

ERRORS: These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Seed Burial. (p89).

Sophora prostrata.

Days	Surface +/-	5mm +/-	10mm +/-	20mm +/-	50mm +/-	100mm +/-
6	0.0464	----------	----------	0.0332	----------	----------
12	0.0693	0.0332	----------	0.0332	0.0895	----------
18	0.0926	0.0332	----------	0.0332	0.0928	----------
24	0.0822	0.0464	0.0693	0.0557	0.0822	----------
30	0.0851	0.0693	0.0875	0.0875	0.0822	----------
36	0.0875	0.0786	0.0895	0.0875	0.0822	----------
42	0.0920	0.0786	0.0910	0.0875	0.0822	----------
48	0.0910	0.0786	0.0910	0.0875	0.0822	----------
54	0.0910	0.0786	0.0910	0.0875	0.0822	----------
60	0.0910	0.0786	0.0910	0.0875	0.0822	----------

ERRORS: These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Seed Burial (p88).

Sophora microphylla.

Days	Surface +/-	5mm +/-	10mm +/-	20mm +/-	50mm +/-	100mm +/-
6	0.0100	----------	----------	----------	0.0142	----------
12	0.0174	0.0142	0.0100	----------	0.0499	0.0142
18	0.0441	0.0314	0.0245	----------	0.0500	0.0142
24	0.0500	0.0485	0.0456	0.0479	0.0496	0.0142
30	0.0502	0.0501	0.0502	0.0502	0.0496	0.0142
36	0.0502	0.0498	0.0502	0.0494	0.0496	0.0142
42	0.0502	0.0498	0.0502	0.0492	0.0496	0.0174
48	0.0482	0.0490	0.0502	0.0494	0.0496	0.0224
54	0.0476	0.0490	0.0502	0.0494	0.0496	0.0224
60	0.0476	0.0490	0.0502	0.0494	0.0496	0.0224

ERRORS:. These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Light/Temperature Interactions. (p93).

Sophora prostrata. 12 hrs light 12 hrs darkness.

Days	10°C +/-	20°C +/-	30°C +/-
3	0.0332	0.0464	----------
6	0.0464	0.0743	----------
9	0.0557	0.0910	0.0332
12	0.0786	0.0822	0.0434
15	0.0851	0.0743	0.0434
18	0.0895	0.0631	0.0434
21	0.0920	0.0464	0.0434
24	0.0928	0.0464	0.0434
27	0.0928	0.0464	0.0434
30	0.0920	0.0464	0.0434

ERRORS: These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Light/Temperature Interactions. (P93).

Sophora prostrata. Dark

Days	10°C +/-	20°C +/-	30°C +/-
3	0.0631	0.0631	0.0332
6	0.0786	0.0786	0.0693
9	0.0910	0.0910	0.0693
12	0.0920	0.0920	0.0693
15	0.0743	0.0743	0.0693
18	0.0571	0.0577	0.0693
21	0.0464	0.0464	0.0693
24	0.0464	0.0464	0.0693
27	0.0464	0.0464	0.0693
30	0.0464	0.0464	0.0693

ERRORS: These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Light/Temperature Interactions. (p92).

S. microphylla, 12 hrs light 12 hrs darkness.

Days	10°C +/-	20°C +/-	30°C +/-
3	----------	----------	0.0174
6	----------	0.0100	0.0224
9	----------	0.0378	0.0348
12	0.0200	0.0429	0.0378
15	0.0423	0.0469	0.0416
18	0.0473	0.0488	0.0429
21	0.0500	0.0498	0.0435
24	0.0502	0.0502	0.0435
27	0.0498	0.0502	0.0435
30	0.0490	0.0501	0.0435

ERRORS: These errors are expressed in digital proportions. For example an error of +/- 0.0332 could be expressed as 3.32%.

Light/Temperature Interactions. (p92).

S. microphylla. Dark.

Days	10°C +/-	20°C +/-	30°C +/-
3	0.0100	----------	----------
6	0.0100	0.0200	----------
9	0.0224	0.0348	0.0100
12	0.0314	0.0435	0.0100
15	0.0441	0.0465	0.0100
18	0.0479	0.0501	0.0100
21	0.0488	0.0502	0.0100
24	0.0500	0.0500	0.0100
27	0.0501	0.0499	0.0100
30	0.0485	0.0496	0.0100